MATERNAL ENVIRONMENTAL FACTORS INFLUENCING KOCHIA (KOCHIA SCOPARIA) SEED CHARACTERISTICS

by

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Abstract

A better understanding of kochia (*Kochia scoparia*) seed characteristics is necessary for long term management of this increasingly troublesome weed. The objectives were to evaluate maternal environmental factors influencing kochia seed produced in the field and to document variability in dormancy and seed viability produced within a single kochia plant grown in the greenhouse or field. Field experiments were conducted in 2012 and 2013 at the Kansas State Agricultural Research Center in Hays. Two different kochia biotypes from Hays were planted with and without five canopy types, namely corn, soybean, grain sorghum, wheat stubble, and kochia plants. A greenhouse experiment with two generations (F1 and F2) of self-pollination was conducted with the same kochia biotypes. Date of initial flowering and final plant heights were recorded. Plants were harvested when seed was mature and divided into three equal parts (top, middle, and bottom). Seeds were cold treated or not, and approximately 50 seeds were placed in petri dishes with water for germination counts taken over six weeks. Viability of remaining seeds were then tested. For field-grown kochia, plants were taller in corn, sorghum and weedy canopies compared to the absence of a canopy. Seed germination from field grown kochia ranged between 77 and 100% for both treatments. There was reduced germination in the presence of a weedy canopy for both treatments and biotypes (77 to 82%) compared to the absence (93 to 99%), with an increase in hard viable seed in the presence of weedy canopy (5 to 14%). In the greenhouse, the F2 generation produced more immediately germinable seed compared to the F1 generation which had more seed with delayed germinability. Seed from bottom third of F1 and F2 plants had greater total germination (73 and 70%, respectively) compared to the middle (61 and 65%) and top (50 and 59%) thirds of the plant. There was a maternal environmental effect on kochia seed characteristics with implications on generating persistent seed for the future seedbank.

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Dedication

I would like to dedicate this thesis to my wife Megan for all your support and understanding and to my mother Carol, and my sister Laura for your moral support and to my late father Martin.

Chapter 1 - Review of Literature

Biology

Kochia (*Kochia scoparia* (L.) Schrad) is an annual broadleaf weed found throughout crop and ruderal areas of the Canadian Prairies and the Great Plains of the United States (Wiese 1970; Nussbaum et al. 1985; Leeson et al. 2005; Friesen et al. 2009). It is a member of the *Chenopodiaceae* or goosefoot family, native to Eurasia and was introduced to the Americas in the mid to late 1800's as an ornamental that subsequently escaped cultivation and formed naturalized populations (Georgia 1914; Scoggan 1957). Kochia is a C_4 summer annual herbaceous dicot plant that thrives in hot and dry environments due to its ability to germinate at low soil temperatures, grow rapidly (Evetts and Burnside 1972; Schwinghamer and Van Acker 2008), and its tolerance to heat, drought (Pafford and Wiese 1964; Coxworth et al. 1969), and saline soils (Weatherspoon and Schweizer 1970).

Kochia is one of the first weed species to germinate early in the spring. Kochia germinates from the soil surface, and even shallow seed burial (20 mm) can reduce kochia emergence to 27% (Schwinghamer and Van Acker 2008). Kochia seed germination is favored by cool to moderate temperatures of 10 to 20 C and light is not required for germination (Everitt et al. 1983). Early emergence offers kochia distinct survival and competitive advantages in cropping systems (Evetts and Burnside 1972) by exploiting the spring soil moisture in arid and semiarid regions (Eberlein and Fore 1984). Although kochia germinates early, additional flushes can occur sporadically throughout the growing season, inevitably following post-emergence herbicide applications, resulting in substantial uncontrolled populations (Mickelson et al. 2004).

Kochia plant structure is highly variable depending on its maternal environment. When grown in competition with other plants, kochia is erect and may grow up to 2 meters tall; when grown without competition, it assumes a more bushy habit and typically grows to approximately 1 meter tall (Eberlein and Fore 1984) and 1 meter in diameter. The rapid growth of kochia can result in significant yield losses in many cropping systems as well as reduce harvesting efficiency due to its ability to remain green long after normal harvest dates for crops such as wheat and soybean (Friesen et al. 2009).

Kochia typically flowers about 8 to 10 weeks after early spring emergence (Thill and Mallory-Smith 1996). It is a short-day plant that initiates flowering when the light period is less than its critical period of 13 to 15 hours (Eberlein and Fore 1984). Flowers on the plant are found in clusters in the axils of upper leaves and in terminal spikes (Eberlein and Fore 1984). Kochia produces a protogynous flower where the stigma emerges, and is able to accept foreign pollen, and then deteriorates before pollen is shed and anther dehiscence which prevents self-pollination within the same flower (Stallings et al. 1995). This type of flowering system and the copious amount of pollen production for extended periods (Mulugeta 1991) is an indication that the plant species is naturally highly outcrossing (Friesen et al. 2009) but also allows for self-pollination within the same plant.

Kochia is known as a very prolific seed producer (Nussbaum et al. 1985), but seed production per plant is dependent upon stand density and intra- and interspecific competition (Becker 1978; Mulugeta 1991). Kochia has been documented to produce up to 30,000 seeds per plant (Stallings et al. 1995) but in a summary of small-plot studies maximum seed production ranges from 15,000 to 25,000 seeds per plant (Watson et al. 2001). Kochia plants grown without competition can produce over 330,000 seeds per plant (A. R. Esser, unpubl. data). Kochia 1000-seed weights vary widely and have been documented to range from 0.47 g (Nussbaum et al. 1985) to 0.85 g (Stevens 1932) to 1.2 g (Liebman and Sundberg 2006). The dispersal of kochia seed is aided by its tumbleweed-like structure, such that as the plant matures, seed rain can occur around the mother plant followed by stem breakage after senescence that allows the whole aboveground plant to tumble freely spreading seed for long distances (Becker 1978). Kochia is known to have the highest rate of spread among alien weeds in the western United States from 1880 to 1980 due to its tumbleweed mode of seed dispersal (Forcella 1985).

Kochia seeds are nearly oval and measure 1.5 to 2.0 mm long, and are often enclosed by a fragile star-shaped hull (Friesen et al. 2009). Kochia seeds can overwinter on the plant or on or below the soil surface (Georgia 1914; Frankton and Mulligan 1987). It is often suggested that kochia seed generally does not exhibit a high degree of innate dormancy (Dyer et al. 1993; Everitt et al. 1983; Thompson et al. 1994). Innate dormancy is seed that are born dormant on the plant (Harper 1959). Kochia seed is not expected to have physical dormancy but physiologically-based dormancy which is the most abundant dormancy class ‘in the field’ (Finch-Savage and Leubner-Metzger 2006) and prevents germination until a chemical change takes place in the seed (Fenner and Thompson 2005). Mature kochia seed is readily germinable, and this occurs rapidly under favorable conditions (Zorner et al. 1984), with the radicle commonly penetrating its seed coat within 24 hours (Steppuhn and Wall 1993). Generally kochia seed is relatively short lived in the soil with seed bank longevity lasting between one to two years. In the absence of seed return, the germinable end-of-season kochia seed bank is typically less than 10% of total kochia seedlings that emerges throughout the growing season (Schwinghamer and Van Acker 2008). This means kochia populations are seed limited and if seed return is prevented populations will not be persistent (Schwinghamer and Van Acker 2008). Knowledge of weed seed emergence patterns and seedbank persistence can be used to suggest cultural practices, such as optimal planting dates, that favor crops over weeds (Nazarko et al. 2005).

Crop height can increase crop competitive ability and reduce growth and seed production by weedy species (Lehnhoff et al. 2013). Taller varieties of winter wheat (*Triticum aestivum* L.) suppressed weed seed production of downy brome (*Bromus tectorum* L.) compared to shorter varieties (Blackshaw 1994). The environment in which the mother plant grows (maternal environmental) during seed production and maturation can influence seed viability, seed dormancy, and subsequent survival in the seedbank (Baskin and Baskin 1998). Seeds formed under severe drought, high temperatures, or short days generally have low levels of seed dormancy (Fenner 1991). Differences in seed dormancy among populations have been documented in many instances. Common waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] seed collected from agricultural fields in Iowa had higher levels of seed dormancy when compared to seeds from nonagricultural fields (Leon and Owen 2006). Differences in seed dormancy can also occur within individual plants where seeds maturing in different positions on the plant may experience different environmental conditions (Baskin and Baskin 1998), as observed in populations of field pennycress (*Thlaspi arvense* L.), corn spurry (*Spergula arvensis* L.), and wild mustard (*Sinapis arvensis* L.) (Andersson and Milberg 1998). On a given plant, seeds produced in shaded conditions may be immature or may exhibit less dormancy than those exposed to full sunlight (Brainard et al. 2005). Alternatively, seeds produced in shade may have higher dormancy as an adaptive mechanism to avoid germination in light-limited environments (Jha et al. 2010). In contrast with this, Lehnhoff et al. (2013) showed that wild oat (*Avena fatua*) seed produced in the overstory of barley (*Hordeum vulgare*) consistently exhibited increased viability compared to seed produced in the understory. Seed dormancy contributes to weedy species plasticity, conferring the ability to survive in changing environments (Mortimer 1997).

Production of seeds in the same plant with different morphologies or ecological behavior, or somatic seed polymorphism (Harper 1977; Venable 1985), is a frequent characteristic of the *Chenopodiaceae* family (Sorensen 1978). Weed species usually show high levels of polymorphism for adaptive traits like seed germination and seed dormancy (Mortimer 1997). For example, Sbatella and Wilson (2010) showed that a kochia biotype that predominated where isoxaflutole was applied as a pre-emergence herbicide had elevated levels of seed dormancy that required higher alternating temperatures to release dormancy than an untreated control kochia biotype.

Herbicide Resistance

Kochia has developed resistance to four different herbicide modes of action including acetolactate synthase (ALS) inhibitors, EPSPS (5-enolpyruvyl-shikimate-3-phosphate synthase) inhibitors, synthetic auxins, and photosystem II inhibitors (Heap 2014; Peterson 1999; Thompson et al. 1994; Waite et al. 2013). The ability of kochia to develop herbicide resistance so quickly and easily when compared to other weeds is possibly a function of several factors. Kochia has difficult-to-wet leaf characteristics that may decrease herbicide efficacy (Friesen et al. 2009). Kochia leaves are generally pubescent with crystalline epicuticular wax, which may result in herbicide spray droplets suspended above the cuticle and consequently reduced absorption (Harbour et al. 2003). Kochia growth stage and herbicide dose markedly affect herbicide efficacy with a decline in control as plant size increases (Friesen et al. 2009). Kochia has high genetic diversity among and within populations which is a contributing factor for selection of herbicide-resistant individuals (Mengistu and Messersmith 2002), as well as wind-mediated pollen movement and protogynous flowering which results in a high degree of outcrossing (Guttieri and Eberlein 1998), and leads to increasingly more herbicide-resistant populations of this species (Thompson et al. 1994).

Herbicide resistant kochia first appeared in Kansas in 1976 to triazine herbicides in cultivated fields (Peterson 1999). Resistance in kochia to triazine herbicides usually is endowed by a point mutation in the chloroplast *psb*A gene, resulting in a substitution of glycine for serine at residue 264 (Mengistu et al. 2005). Similarly, ALS-inhibitor resistance in kochia usually is endowed by a single point mutation in the ALS nuclear gene and is inherited as a dominant or semidominant trait and is controlled by a single nuclear gene (Primiani et al. 1990). Chlorsulfuron resistant kochia was confirmed in Kansas wheat fields in 1987 (Thompson et al. 1994). More recently, in 2007, glyphosate resistant kochia was confirmed in Kansas in multiple cropping systems such as corn, cotton and soybeans (Waite et al. 2013). Resistance to glyphosate in kochia is from gene amplification of EPSPS (Wiersma 2012).

Kochia is an increasingly problematic weed in the Great Plains region of North America and there are increasingly more herbicide-resistant populations of this species (Thompson et al. 1994; Waite et al. 2013). Kochia seed characteristic responses to canopy types are not well known. However, knowing that maternal environment can affect seed dormancy characteristics, more information is needed regarding kochia seed viability and dormancy under common crop canopies in the Great Plains region to help aid in designing future weed management practices. The research objectives were 1) to evaluate maternal environmental factors influencing kochia seed produced in the field 2) to document variability in dormancy and seed viability produced within a single kochia plant grown in the greenhouse or field, and 3) to evaluate the response of several kochia biotypes to field use rates of glyphosate, atrazine, and chlorsulfuron herbicides.

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Chapter 2 - Kochia Seed Characteristics in the Greenhouse and Under Different Canopy Types in the Field

ABSTRACT

A better understanding of kochia (*Kochia scoparia*) seed dynamics is necessary for long term management of this increasingly troublesome weed. The objective was to evaluate maternal environmental effects on kochia growth in the field and document its variability in dormancy and viability of seed produced within a single kochia plant in the greenhouse and field. Field experiments were conducted in 2012 and 2013 at the Kansas State Agricultural Research Center in Hays. Two kochia seed biotypes (cropland and non-cropland) from Hays, KS were planted with and without five different canopy types, in a split plot RCBD, to mimic a typical environment in which kochia is found in the Great Plains. Canopies included corn, soybean, grain sorghum, wheat stubble, and kochia plants. A greenhouse experiment was conducted with the same two kochia seed biotypes from Hays. Kochia plants were grown and limited to self-pollination for two cycles. For both field and greenhouse experiments initial flowering date and final plant heights were recorded. Plants were harvested when seed was mature and divided into three equal parts (top, middle and bottom). Seeds were removed from each section and a germination assessment was conducted with two treatments (non-cold and cold treatments). Subsets of approximately 50 seeds per plant section per petri dish with 10 mL water were placed in a growth chamber with 12 h light: dark at 20:10 C and germination counts were taken for six weeks. A viability assessment was then conducted three months later to test the viability of seeds remaining in the petri dishes. For the field experiment observed kochia plant heights at the end of the growing season were taller in the presence of corn (76 cm), stubble (57 cm), and grain sorghum (75 cm) canopies compared to in the absence of those crop canopies for corn (59 cm), stubble (45 cm), and grain sorghum (57 cm). Germination from field grown kochia ranged between 77 and 100% for both treatments and there were interactions with presence and absence of canopy and kochia biotype. Regardless of the cycle, greenhouse grown kochia seed that came from the bottom third of the plant had greater germination percentages than the middle and top sections. There was a maternal environmental effect on kochia seed characteristics with implications on future seedbank life.

INTRODUCTION

Kochia (*Kochia scoparia* (L.) Schrad) is an annual broadleaf weed that thrives in arid and semiarid climates due to its ability to germinate at low soil temperatures, grow rapidly (Evetts and Burnside 1972; Schwinghamer and Van Acker 2008), and its tolerance to heat, drought (Pafford and Wiese 1964; Coxworth et al. 1969), and saline soils (Weatherspoon and Schwiezer 1970). Kochia is self-compatible and produces protogynous flowers where the stigmas emerge before anther development (Guttieri et al. 1995; Stallings et al. 1995). Kochia plant structure is highly variable depending on its maternal environment. When grown in competition with other plants, kochia is erect and may grow up to 2 meters tall; when grown without competition, it assumes a more bushy habit and typically grows to approximately 1 meter tall (Eberlein and Fore 1984).

Kochia is known as a very prolific seed producer (Nussbaum et al. 1985) where, seed production is dependent upon stand density and intra- and interspecific competition (Becker 1978; Mulugeta 1991). Kochia has been documented to produce up to 30,000 seeds per plant (Stallings et al. 1995) but in a summary of small-plot studies maximum seed production ranged from 15,000 to 25,000 seeds per plant (Watson et al. 2001). Kochia 1000-seed weights vary widely and have been documented to range from 0.47 g (Nussbaum et al. 1985) to 1.2 g (Liebman and Sundberg 2006). The dispersal of kochia seed is aided by its tumbleweed-like structure, such that when the plant matures seed rain can occur around the mother plant and then stem breakage after senescence allows the whole aboveground plant to tumble freely spreading seed for long distances (Becker 1978).

Kochia seeds can overwinter on the plant or on or below the soil surface (Georgia 1914; Frankton and Mulligan 1987). It is often suggested that kochia seed generally does not exhibit a high degree of innate dormancy (Dyer et al. 1993; Everitt et al. 1983; Thompson et al. 1994). Mature kochia seed is readily germinable, and occurs rapidly under favorable conditions (Zorner et al. 1984), with the radicle commonly penetrating its seed coat within 24 hours (Steppuhn and Wall 1993). Kochia seed germination is favored by cool to moderate temperatures of 10 to 20 C and light is not required for germination (Everitt et al. 1983). Kochia populations are seed-limited, with seed bank longevity lasting between one to two years, and if seed return is prevented populations will not be persistent (Schwinghamer and Van Acker 2008).

Crop height can increase crop competitive ability and reduce growth and seed production by weedy species (Lehnhoff et al. 2013). The environment in which the mother plant grows (maternal environmental) during seed production and maturation can influence seed viability, seed dormancy, and survival in the seedbank (Baskin and Baskin 1998). Differences in seed dormancy among populations have been documented in many instances as well as differences within individual plants where seeds maturing in different positions on the plant may experience different environmental conditions (Baskin and Baskin 1998). Seed dormancy contributes to weedy species plasticity, conferring the ability to survive in changing environments (Mortimer 1997). Production of seeds in the same plant with different morphologies or ecological behavior is referred to as somatic seed polymorphism (Harper 1977; Venable 1985). Weed species usually show high levels of polymorphism for adaptive traits like seed germination and seed dormancy (Mortimer 1997).

Maternal environmental factors influencing response of kochia seed to a crop canopy are not well known. However, knowing that maternal environment can affect seed dormancy characteristics, more information is needed regarding levels of kochia seed viability and dormancy under common crop canopies in the Great Plains region to help aid in designing future weed management practices. The research objectives were 1) to evaluate maternal environmental factors influencing kochia seed produced in the field and 2) to document variability in dormancy and seed viability produced within a single kochia plant grown in the greenhouse or field.

MATERIALS AND METHODS

Greenhouse Experiment

This included two cycles of self-pollination, to create F1 and F2 seed generations, to evaluate the potential change in seed characteristics from selfed individual kochia plants. Two kochia seed biotypes from Hays, KS were collected in 2011 from a field edge near a fencerow (cropland) in a cropping system and next to silos (non-cropland) and stored at room temperature until planting. The first cycle was started in January in 2012 at Department of Agronomy greenhouses at Kansas State University, Manhattan, KS. All kochia plants were grown in a greenhouse with a 12:12 day/night cycle at 27:22 ±2 C, respectively. Seed from each biotype were placed on the soil surface for germination and establishment in separate 50 by 35 by 9 cm flats filled with 2.2 kg commercial potting mix (Metro-mix 360)[1]. Eighteen days after seeding 55 plants from each population were transplanted into 1.5 L pots with 460 g commercial potting mix to be grown to maturity. A sub-sample of five kochia plants from each population was taken 31 days after the first transplanting date. These plants were transplanted into larger 8 L pots with 2.4 kg of commercial potting mix and grown to maturity. Plants were watered as needed and fertilized 15 and 26 days after transplanting with water soluble all-purpose plant food (Scotts Miracle-Gro (24-8-16))[2] at 4.0g/L prior to stigma emergence. After first observation of stigma emergence plants were limited to self-pollination using pollination bags to inhibit outcrossing and flowering date was recorded as the first cycle of self-pollination (F1). Kochia were grown until seed was mature and harvested by breaking the stem off at soil surface. Final plant heights were taken and plants were then split into three equal parts based on total plant height (top, middle, and bottom) and branch number was recorded for each section (data not shown). Seeds were harvested from each section and separated from plant parts using an air column separator (South Dakota seedblower)[3] before performing a germination assay (described below).

Parent seed treatments from the F1 generation were non-cold treatment (NCT) where seeds taken directly off the plant and cold treatment (CT) where seeds were placed in a freezer set at -4 C for six weeks to simulate an overwintering effect. To generate F2 plants, seeds from the F1 generation that had been exposed to these two different seed treatments and had exhibited varying rates of germination based on germination assay (described below) were selected. Kochia seeds from four cropland and six non-cropland biotypes with the NCT were started in December of 2012 and CT seed were started in January of 2013. Seed were placed on the soil surface for germination in 28 by 19.5 by 6 cm flats with 480 g of commercial potting mix. Five plants from each selected plant section for the NCT were transplanted 26 days after placement in flats except for one population which had delayed germination and those were transplanted between 31 and 48 days after seed placement in flats. Seedlings from CT seeds were transplanted 30 days after placement in flats. All kochia plants were transplanted into 1.5 L pots with 420 g of commercial potting mix. Plants were watered as needed and fertilized once with water soluble all-purpose plant food at 4.0g/L prior to stigma emergence. After first stigma emergence plants were limited to self-pollination using pollination bags and flowering date was recorded as the second cycle of self-pollination (F2). Plants were grown until seed was mature and harvested by breaking the stem off at the soil surface. Final plant heights were taken and were then split into three equal parts based on height (top, middle, and bottom) and branch number was recorded for each section (data not shown). Seeds were harvested and separated from plant parts using an air column separator before performing the germination assay (described below).

Field Experiments

Field experiments were conducted at the Kansas State Agricultural Research Center in Hays, KS in 2012 and 2013 under no-till systems. In 2012 the field was a Roxbury silt loam soil with 32, 52 and 16% sand, silt and clay respectively. The previous crops were fallow in 2010 and winter wheat in 2011. At time of planting there was approximately 70% standing wheat residue. In 2013 the field was a Crete silty clay loam soil with 16, 53, and 31% sand, silt, and clay respectively. The previous crops were fallow in 2011 and winter wheat in 2012. At time of planting there was 50 to 70% standing wheat residue. For both years of the field experiment previous wheat crop had been planted on 25 cm row spacing at a seeding rate of 67 kg/ha and stubble height at time of establishing this experiment was between 20 and 25 cm. Glyphosate (Roundup WeatherMax) was applied at 868 g ae/ha prior to crop planting both years to remove the existing weed community present in the field.

Kochia seeds were planted with and without five different canopy types to mimic a typical environment in which kochia is found. The field experiment utilized a split-plot randomized complete block design with the main plot being canopy type, with a factorial arrangement of presence or absence of that canopy and the cropland or non-cropland kochia biotypes. The five canopy types were corn, soybean, grain sorghum, weedy (kochia biotype collected in Stockton, KS in 2011), and wheat stubble. Plots were 4 m long by 3 m wide and each treatment was replicated four times. All crops were planted on 76 cm row spacing using a six-row vacuum planter (Monosem)[4]. Seed variety was selected based on environment and in 2012 corn was Dekalb 52-59, grain sorghum was Dekalb 36-06 and soybean was Northrup King S33-K5RR while in 2013 corn was Dekalb 52-19, grain sorghum was Dekalb 37-07 and soybean was Asgrow 2933. The two kochia seed biotypes and corresponding canopies were planted on the same dates. In the first year, corn, weedy, and wheat stubble plots were established on April 25, 2012 along with 90 kg N/ha on the corn plots. Weedy plots in the presence of canopy type were established by overseeding with the kochia biotype from Stockton, KS and allowing existing weed community present to emerge throughout the growing season. Stubble plots in the presence of canopy type were left undisturbed while in the absence of canopy type winter wheat stubble was manually removed from the plot leaving a bare soil surface. This was followed by an application of 34 kg N/ha applied to the entire study on April 26, 2012. On May 11, 2012 soybean and grain sorghum plots were planted with an application of 45 kg N/ha on the grain sorghum plots as a starter fertilizer. In the second year fertilizer was placed in the corn plots at 60 kg N/ha on April 16, 2013, followed by 60 kg N/ha on the rest of the plots on April 30, 2013. Corn, weedy, and wheat stubble plots were established on May 16, 2013 with 48 kg N/ha placed in corn plots at time of planting. Weedy plots in the presence of canopy type were established by overseeding with the kochia biotype from Stockton, KS and allowing existing weed community present to emerge throughout the growing season. Stubble plots in the presence of a canopy were left undisturbed while in the absence of a canopy winter wheat stubble was manually removed from the plot leaving a bare soil surface. On June 3, 2013 soybean and grain sorghum plots were planted with an application of 48 kg N/ha on the grain sorghum plots. All sources of nitrogen were urea ammonium nitrate (28-0-0). Following planting in 2013 a chicken wire fence was set up around the experiment to reduce herbivory by deer and rabbits.

In 2012, the cropland and non-cropland kochia biotypes were seed collected in 2011 from Hays, KS, while in 2013 one plant of each kochia biotype was selected from the field experiment in 2012. Kochia seeds were placed in all plots at time of crop planting in ten spots equally spaced approximately 1 m apart. A suspension gel, which consisted of mixing 12 g of Laponite RD®[5] with 1 L water (Nordby and Hartzler 2004), was utilized to facilitate placement and germination of kochia seed. A set of 100 seeds were placed in 100 mL of suspension gel and homogenized. A 12mL syringe was used to place 10mL of solution containing approximately 10 seeds on the soil surface. After kochia germination seedlings were thinned to one plant per location to establish ten equally spaced plants per plot with data collection focused on four centrally located kochia plants.

Kochia plant heights and crop growth stages were measured weekly throughout the growing season and other weeds in the plots were removed, using a hand hoe as needed, except in the presence of weedy canopy plots. Flowering date was documented for the four centrally located kochia plants in each plot. Final kochia plant heights were documented when seed was mature and up to four plants were harvested from each plot by cutting plant off at soil surface. The plants were split into three equal parts (top, middle, and bottom) by total height measurement at time of harvest. Branch number was recorded for each section (data not shown) and seed was harvested and cleaned using an air column separator. Seed weights were taken based on the weight of 100 seeds and multiplied by 10 to get 1000-seed weights. Total seed production was calculated for each plant section and whole plant by dividing total seed weight by 100 seed weight times 100.

Final height data for field-grown kochia were pooled over both years and were analyzed using PROC MIXED (SAS 9.2)[6] across canopy type, presence or absence of canopy, kochia biotype, and their interactions. For field-grown kochia, seed weights, seed production per plant section, and total seed production per plant were pooled over both years and data were analyzed using PROC MIXED across canopy type, presence or absence of canopy, kochia biotype, position on the plant, and their interactions. Figures were created using SigmaPlot v. 12.3[7].

Germination Assessment

A germination assessment was conducted on seeds collected from top, middle, and bottom sections of the plant from the F1 and F2 generations in the greenhouse experiment and from 2012 seed harvested from the field experiment. Seeds from each section were separated into two equal parts for the two treatments which were non-cold treatment (NCT) and cold treatment (CT). The NCT were seed harvested directly off the plant and the CT seeds were placed in a freezer set at -4 C for six weeks prior to the assessment being conducted. The CT was performed to simulate an overwintering period that seeds might experience under typical field conditions. A subset of approximately 50 seeds from each plant section was placed in a 100 mm by 15 mm petri dish with filter paper and 10 mL of water. Petri dishes were placed in a growth chamber at 12:12 day/night cycle at 20:10 C, respectively. After seeds had germinated, which was defined by the presence of the radicle they were counted and removed. Germination counts were performed for six weeks after being placed into the growth chamber with counts done every day for two weeks, every other day for the following two weeks and every third day for the last two weeks.

A viability assessment was performed approximately two to three months later to classify the remaining seed in petri dishes. Seed in petri dishes were re-wetted with 5mL of water. Three days following re-wetting a press test was performed (Davis et al. 2005) where seeds were classified as hard viable seed, seed that germinated during the wetting period, or soft not viable seed. Thus, all seeds were classified into four types: immediately germinable seed, seed with delayed germinability, hard viable seed, and soft not viable seed.

Final percent germination and viability of greenhouse-grown kochia seed were analyzed using PROC MIXED across kochia biotype, treatment, position on the plant and their interactions. Final percent germination and viability of field-grown kochia seed were analyzed using PROC MIXED across canopy type, presence or absence of canopy, kochia biotype, position on the plant and their interactions. Figures were created using SigmaPlot v.12.

RESULTS AND DISCUSSION

Greenhouse Experiment

In the F1 generation initial flowering dates for kochia plants ranged from 67 to 96 and 60 to 87 days after planting (DAP) for the cropland and non-cropland biotypes respectively. Initial flowering dates for the subsample of plants transplanted into larger pots were later and ranged from 82 to 104 DAP and 67 to 81 DAP for cropland and non-cropland biotypes respectively. There was a significant difference in kochia plant heights with the cropland biotype being taller (134 cm) compared to the non-cropland biotype (107 cm) (Table 2.1). The subsample plants had increased growth and also showed a significant difference with the cropland biotype having taller plants (236 cm) compared to the non-cropland biotype (136 cm) (Table 2.1). Taller plants in the subsample of the cropland were due to the more days before first flower giving the plants more days of vegetative growth.

The number of kochia plants that produced enough seed to conduct a germination assessment from the F1 seed generation was greater for the non-cropland biotype that had 38 plants, compared to the cropland biotype that had 10 plants. As a result of the germination assessment, the non-cropland biotype had 31% and greater immediately germinable seed than the cropland biotype with only 19% (Figure 2.1). Immediately germinable seed was greater in the bottom third of the plant (32%) compared to the middle and top thirds having 24 and 19% respectively, in regards to position on the plant (Figure 2.2). Cold treatment (CT) seed had 33% immediately germinable seed compared to the non-cold treatment (NCT) seed which had 17%

(Figure 2.1). Seeds with delayed germinability were not different between biotypes (Figure 2.1). Differences were still seen with seed from the bottom third of the plant having more seed with delayed germinability at 41% while the middle and top thirds had 37, and 32% respectively (Figure 2.2). The seed with delayed germinability from the NCT seed was 46% while the CT seed was 27% (Figure 2.1). Hard viable seed remaining was greater for the cropland biotype with 7%, compared to the non-cropland biotype with 4% (Figure 2.1). There were no differences with regards to position on the plant for hard viable seed (Figure 2.2). There was however a significant difference in hard viable seed remaining with the CT seed having 7% while the NCT seed had 5% (Figure 2.1). Total percent germination declined as days to first flower was delayed for top, middle, and bottom sections of the plant for both seed treatments (Figure 2.3).

For the F2 generation of the cropland biotype, initial flowering dates ranged from 39 to 92 DAP for the NCT parent seed and 64 to 99 DAP for the CT parent seed treatment. For the F2 generation of the non-cropland biotype initial flowering dates ranged from 55 to 92 DAP for the NCT parent seed and ranged from 56 to 100 DAP for the CT parent seed. Final kochia plant heights showed a significant two-way interaction between kochia biotype and parent seed treatment. Parent seed from the CT cropland biotype had taller plant heights (103 cm) compared to the NCT cropland biotype (83 cm) and both NCT (92 cm) and CT (92 cm) parent seed from the non-cropland biotype (Table 2.2). The number of kochia plants that produced enough seed to conduct the germination assessment was less with the cropland biotype having three NCT and six CT plants compared to 14 NCT and nine CT plants for the non-cropland biotype. Less productive plants in the F2 generation is due to the inbreeding depression that is seen with kochia.

The NCT parent seed had greater germination for both cropland and non-cropland biotypes with 82 and 75% respectively, compared to the CT parent seed for the cropland biotype with 36% germination. The only difference for immediately germinable seed was with the NCT cropland parent seed where CT seeds had greater germination (86%) compared to the NCT seeds (78%) (Figure 2.4). Position on the plant was significant for immediately germinable seed where the bottom third of the plant had greater germination of 69% compared to the middle (63%) and top (57%) thirds (Figure 2.5). Seed with delayed germinability was greater in the cropland biotype with the CT parent seed with NCT seed with 5% and CT seed with 6% compared to all other treatments (Figure 2.4). The percent of hard viable seed was greater with 5% for the CT parent seed for both biotypes compared to 1% for the NCT seed for both biotypes across germination seed treatments (Figure 2.4). There was greater variability seen in total percent germination for the F2 generation with delay in days to first flower (Figure 2.6) compared to the F1 generation which could be attributed to plants in the F2 generation being more homozygous.

The results of the greenhouse experiment showed that regardless of the cycle greenhouse-grown kochia had greater germination from seed harvested from the bottom third of the plant compared to the middle and top thirds. This could be due to the flowering pattern of kochia, which starts on the top of the plant and at the tips of the branches, and then progresses towards the main stem. Also, it could be due to the bagging of individual plants for self-pollination which allows pollen that is shed first near the top of the plant to flow down to the bottom sections of the plant giving it a greater probability for a successful self-pollination and production of viable seed. In the F2 generation fewer plants produced viable seed compared to the F1 generation. This is due to kochia naturally being an outcrossing plant and there being a penalty due to self-pollination. Variability exists within populations of kochia for germination and viability allowing kochia populations to adapt to different conditions.

Field Experiment

The weather in Hays, KS varied between years. Mean temperatures in 2012 were greater from April to July during the vegetative growth period of kochia compared to 2013 (Table A.1). Similar mean temperatures were recorded during the month of August both years during the flowering period for kochia (Table A.1). In 2013 there were warmer temperatures in the months of September and October during the seed maturation period, compared to 2012 (Table A.1). Kochia is a photo-period-sensitive plant that initiates flowering when the light period is less than the critical period, usually between 13 and 15 hr (Bell et al. 1972). Average flowering dates were relatively similar for both years regardless of planting date and in 2012 occured between August 21 and 26 and in 2013 occurred between August 13 and 17. During these flowering dates day length in Hays was between 13 hours 44 minutes and 13 hours 13 minutes. In 2012 the site received 365 mm of rain compared to the 30-year average of 595 mm (Table A.2), and in 2013 the site received 50 mm less than the annual average, but nearly one-third of the year’s precipitation fell during the month of July (Table A.2).

Kochia plant heights through the growing season for both years of the field experiment are presented in Appendix B. Final kochia plant heights in the field had a significant two-way interaction with canopy type and presence or absence of canopy. Final plant heights were taller in the presence of a canopy for corn (76 cm), stubble (57 cm), and grain sorghum (75 cm), compared to in the absence of those canopies at 59, 45 and 57 cm respectively with a LSD (0.05) of 8.3 (Table 2.3). Weedy canopy in 2012 was primarily kochia with small infestations of yellow foxtail (*Setaria viridis*), puncturevine (*Tribulus terrestris*), and longspine sandbur (*Cenchrus longispinus*), while in 2013 the primary weed species was puncturevine throughout the growing season with few kochia establishing from the overseeding and a late flush of Palmer amaranth (*Amaranthus palmeri*) following the July rainfall event. Also, the cropland biotype of kochia had taller final plant heights in the weedy plot at 60 cm compared to the non-cropland biotype which had final heights of 47 cm with a LSD of 8.3 (Table 2.3).

Kochia 1000-seed weights showed a significant three-way interaction among canopy type, presence or absence of canopy, and kochia biotype. Seed weights varied from 0.62 to 0.92 g across all treatments with a LSD of 0.16 (Table 2.4), which was similar to previous studies (Nussbaum et al. 1985; Liebman and Sundberg 2006). There was also a difference of 1000-seed weights among positions on the plant with greater weights in the bottom (0.73 g) and middle (0.74 g) thirds of the plant compared to the top (0.68 g) (Table 2.5). Seed production based on position on the plant showed a two-way interaction between presence or absence of crop canopy and position on the plant. Seed produced on the bottom section of plants in the absence of a canopy had greater seed production (22,785 (SE 1,851)) compared to in the presence of a canopy (8,915 seeds (SE 1,646)) (Figure 2.7). The distribution of seed production by position on the plant correlates with the variability of kochia plant structure, which varied depending on maternal environment. Total seed production per plant showed a significant three-way interaction among canopy type, presence or absence of canopy, and kochia biotype. Seed production ranged from 2,663 to 45,455 seeds per plant across all treatments with a LSD of 25,613 (Table 2.6). In general, kochia plants from plots with the absence of a canopy had greater seed production than in the presence of a canopy for both biotypes (Figure 2.8). In previous small plot research kochia seed production was documented to be between 15,000 and 25,000 seeds per plant (Watson et al. 2005). Maximum seed produced from a single kochia plant was over 330,000 seeds which occurred in the absence of a canopy in 2013. In the presence of a weedy canopy kochia seed production per plant was the lowest among all treatments for cropland (6,629 (SE 5823)) and non-cropland (2,663 (SE 5979)) biotypes (Table 2.6).

Germination for the NCT kochia seed ranged between 81 and 100% across all canopy types, presence or absence of that canopy and kochia biotype. Germination did not differ based on the position of kochia seed on the plant. There was an interaction between presence or absence of canopy and kochia biotype for percent germination and viability in the weedy canopy (Table 2.7). In the absence of a weedy canopy immediately germinable seed were greater for the cropland and non-cropland biotypes with 96 and 95% respectively, compared to the presence of a weedy canopy where immediately germinable seed for the cropland and non-cropland biotypes were 81 and 82% respectively (Table 2.7). The hard viable seed remaining was greater in the presence of a weedy canopy at 6 and 14% for non-cropland and cropland biotypes, respectively compared to 1% in the absence of a weedy canopy for both biotypes (Table 2.7).

Germination for the CT kochia seed ranged between 77 and 100% across all canopy types, presence or absence of that canopy and kochia biotype. Germination of seed from different positions on the plant was not different. There was an interaction for percent germination between the presence or absence of canopy and the kochia biotype for the sorghum and weedy canopies (Table 2.8). In the absence of a grain sorghum canopy the cropland biotype had greater immediately germinable seed with 100% compared to the non-cropland biotype with 87% and in the presence of a grain sorghum canopy, with cropland (91%) and non-cropland (87%) biotypes (Table 2.8). In the absence of a weedy canopy immediately germinable seed was greater for the cropland (93%) and non-cropland (87%) biotypes, compared to immediately germinable seed in the presence of a weedy canopy where the cropland and non-cropland biotypes were 77 and 82% respectively (Table 2.8). There was also an interaction among the presence or absence of a canopy and kochia biotype for the percent viability in the weedy canopy (Table 2.8). The hard viable seed remaining was greater in the presence of a weedy canopy at 5 and 12% for non-cropland and cropland biotypes, respectively, compared to 0% in the absence of a weedy canopy for both biotypes (Table 2.8).

The results show that there was a maternal environmental effect on field-grown kochia with heights in the presence of a canopy being taller than in the absence of a crop canopy. This was due to the competition of kochia with the canopy forcing kochia to alter its growth and grow taller instead of forming a more bushy-type appearance which occurs without competition. This change of plant structure based on maternal environment was also observed in the distribution of seed production per plant section. Total seed production was in general greater in the absence of a canopy at 31,455 seed per plant compared to in the presence of a canopy at 15,143 seed per plant across both biotypes. Seed production per plant was less in the presence of a weedy canopy compared to all other treatments. Seed germination for NCT and CT seed from both cropland and non-cropland biotypes were between 77 and 100% with no differences seen with position of seed on the plant. This is different from previous research by Leon and Owen (2006) showing higher levels of seed dormancy with common waterhemp seed collected in an agricultural source compared to a non-agricultural source. It is also different than research by Lehnhoff et al. (2013) showing wild oat seeds produced in the overstory of barley having greater viability compared to seed produced in the understory. There was a reduction in germination in the presence of a weedy canopy compared to in the absence of one, but seed remaining in the presence of the weedy canopy had more hard viable seed. This could have implications on generating persistent seed for the future seedbank. Results from this study could aid in modeling kochia seed life under various canopy types common to the Great Plains region of North America. Kochia seed is not expected to have physical dormancy but physiologically-based dormancy which prevents germination until a chemical change takes place in the seed (Fenner and Thompson 2005). Future research could use these seeds with varying levels of dormancy to study the possible chemical change that is taking place within the seed.

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Figures and Tables

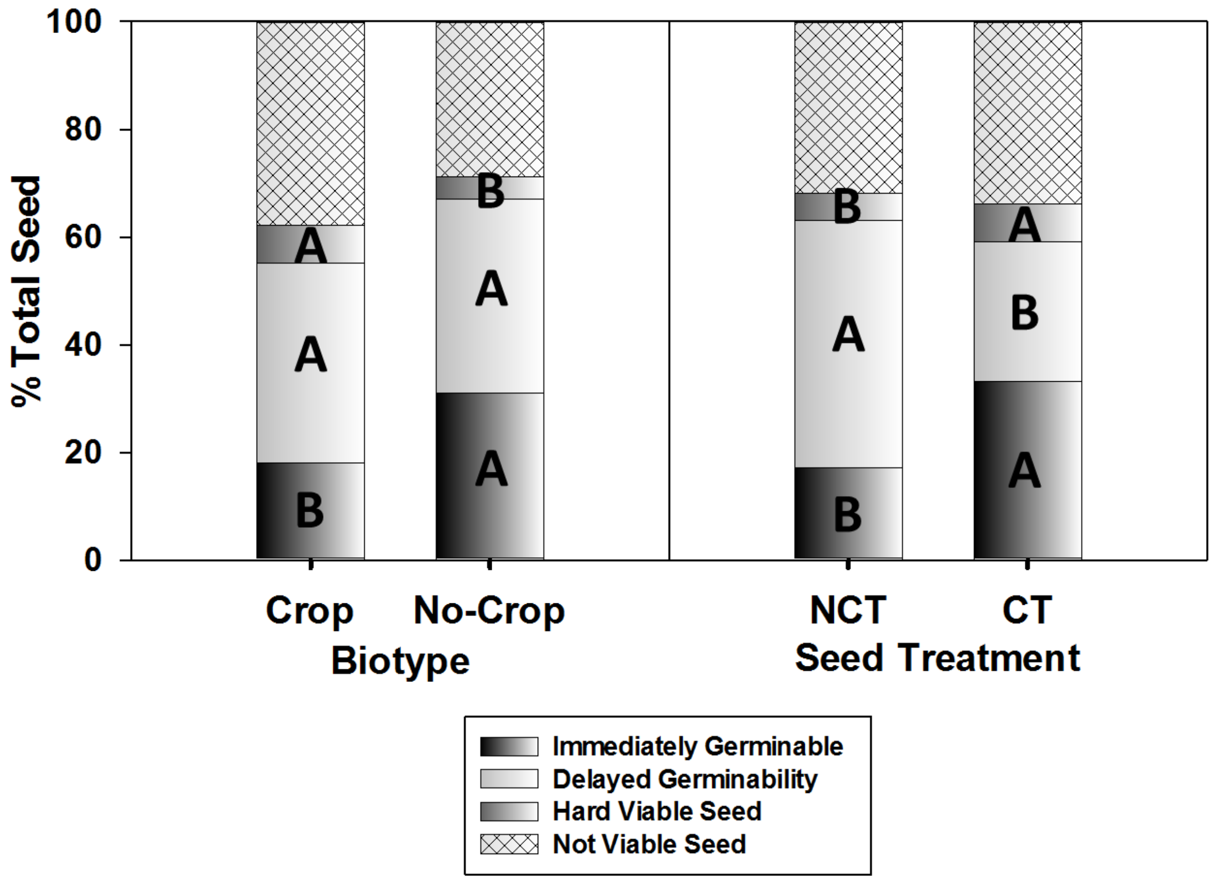

Figure 2.1 Total seed classification for cropland and non-cropland kochia biotypes and non-cold treatment (NCT) and cold treatment (CT) seed treatments for the F1 generation in the greenhouse.

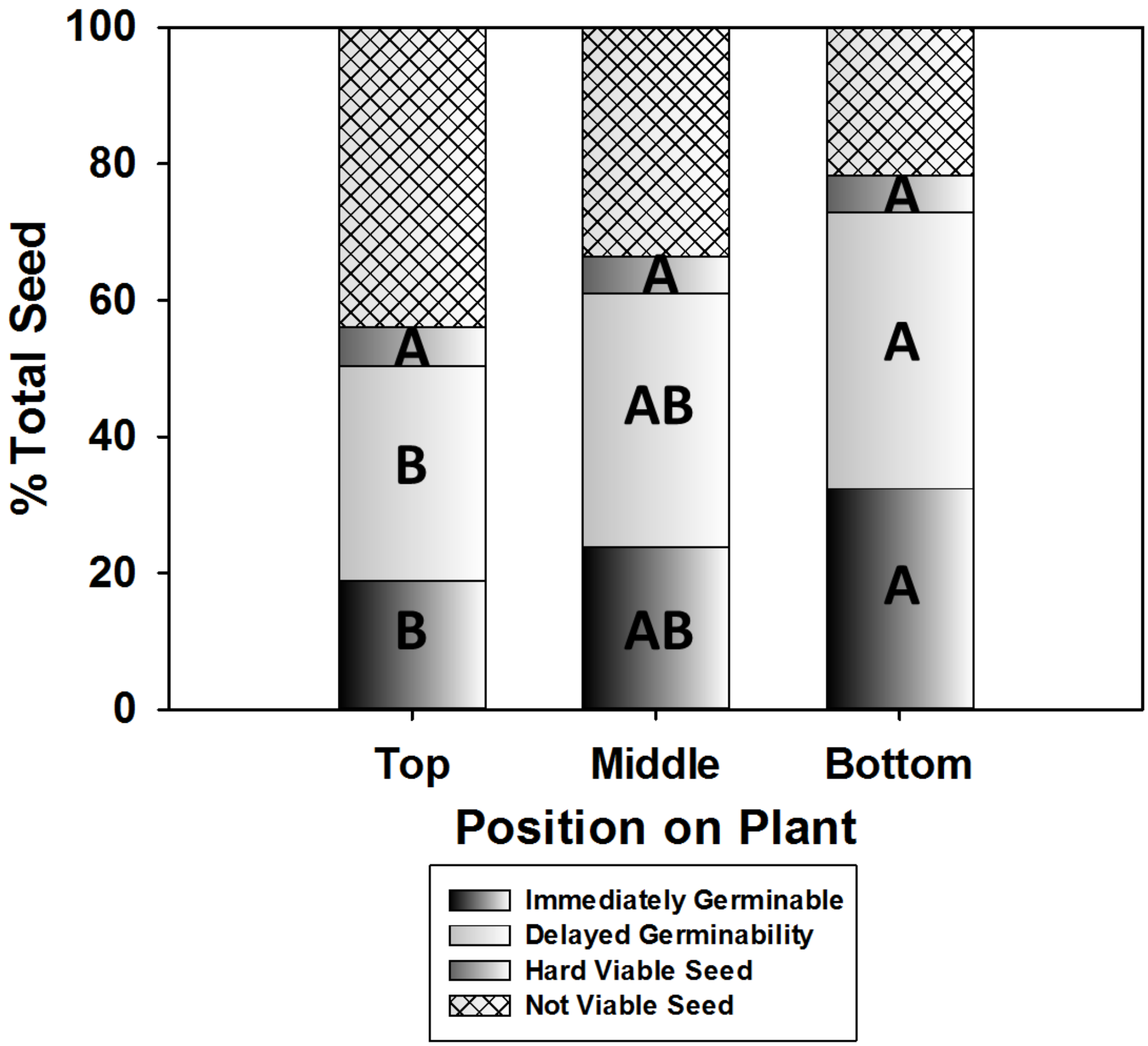

Figure 2.2 Percent of total seed for position on the plant (top, middle, and bottom) for all biotypes and treatments for the F1 generation in the greenhouse.

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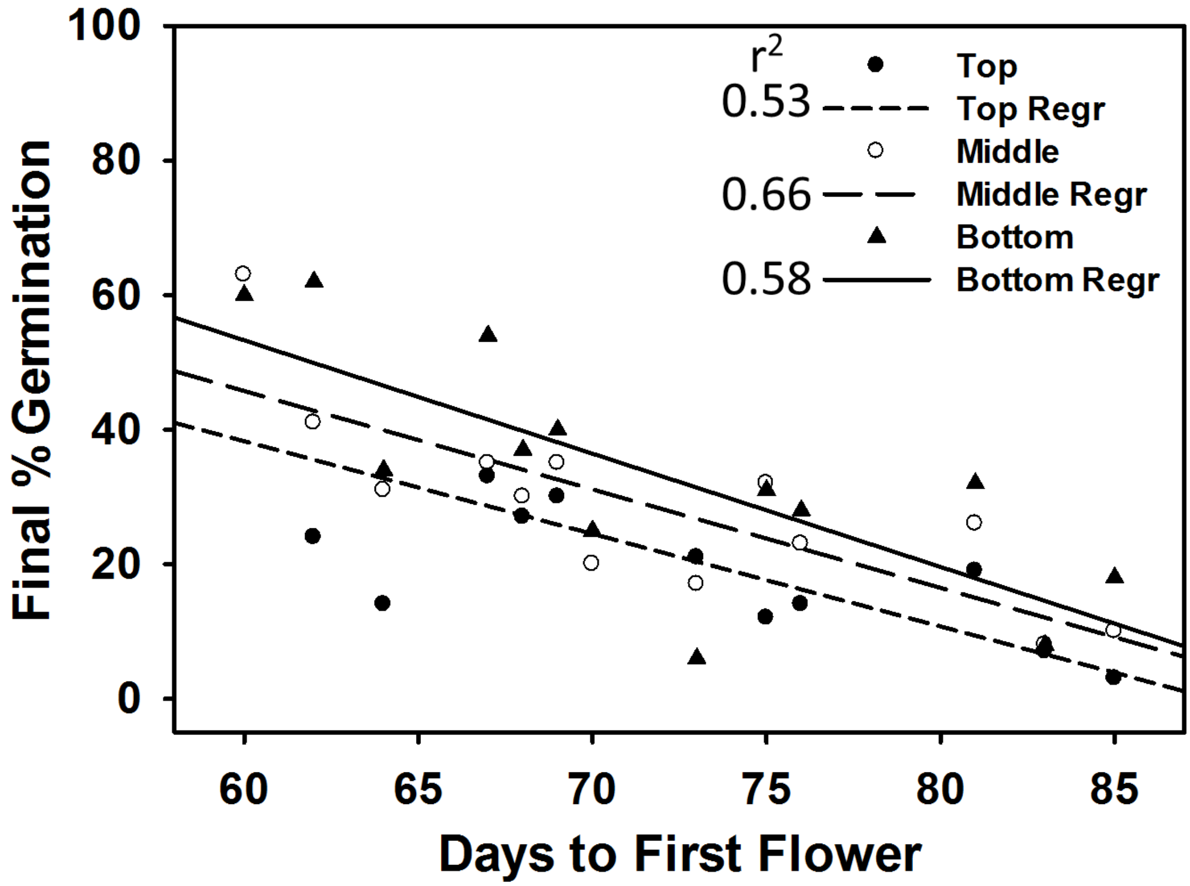

Figure 2.3 Final percent germination of kochia seed from the top, middle and bottom sections of plants corresponding to the days to first flower for the F1 generation in the greenhouse.

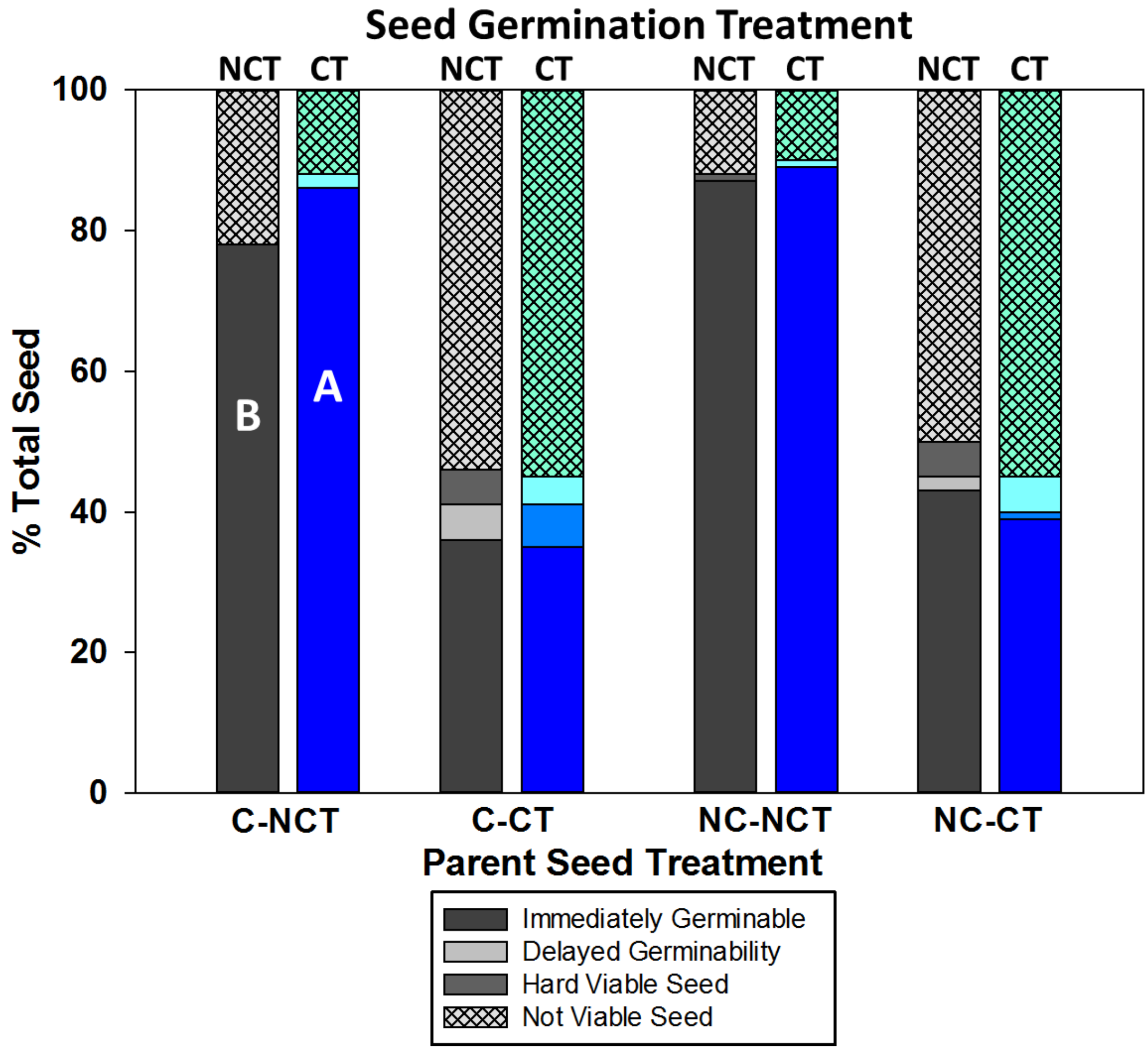

Figure 2.4 Percent of total seed for cropland (C) and non-cropland (NC) kochia biotypes with non-cold treatment (NCT) and cold treatment (CT) parent seed treatments and seed germination treatments for the F2 generation in the greenhouse.

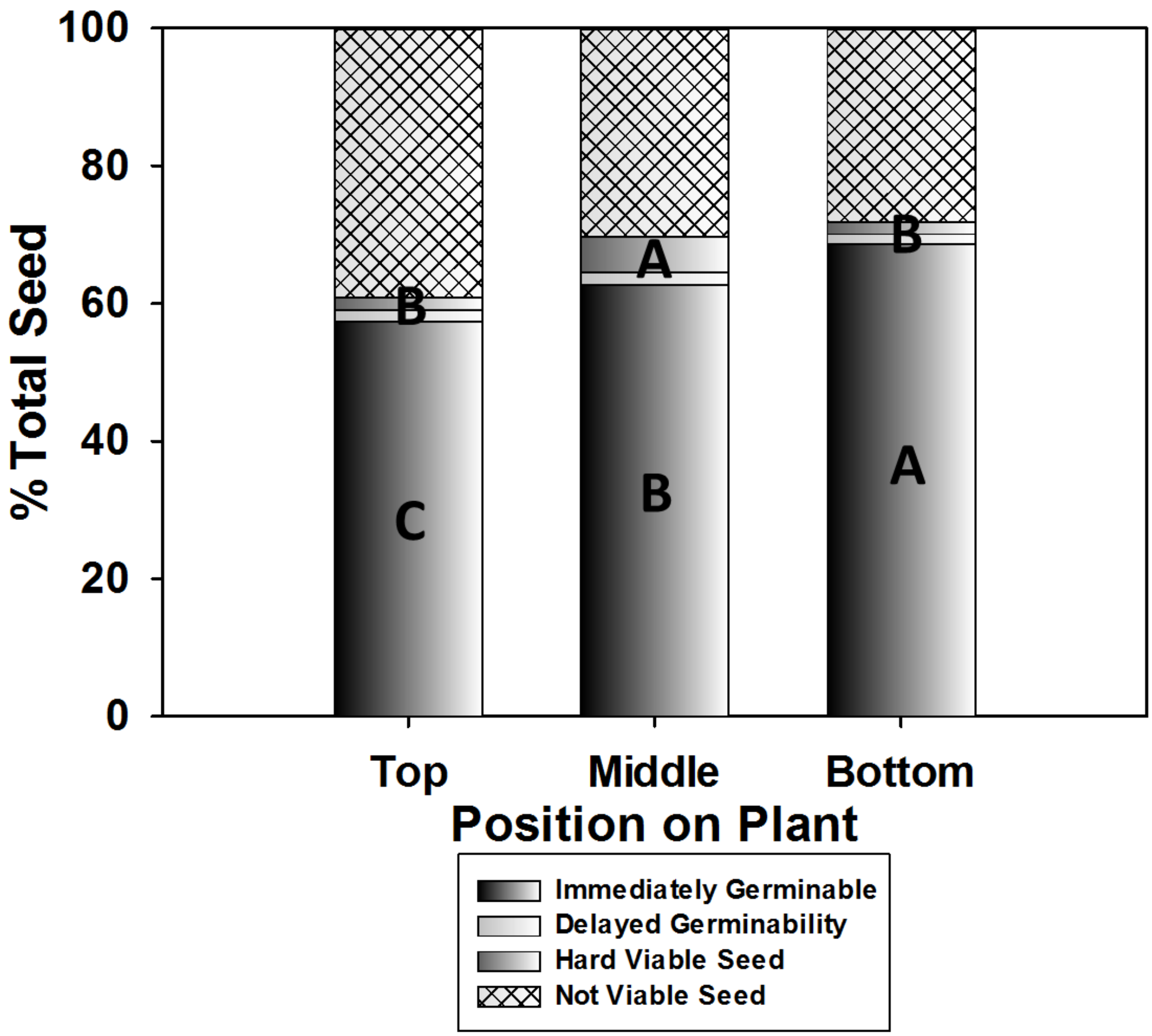

Figure 2.5 Percent of total seed for position on the plant (top, middle, and bottom) for all biotypes and treatments for the F2 generation in the greenhouse.

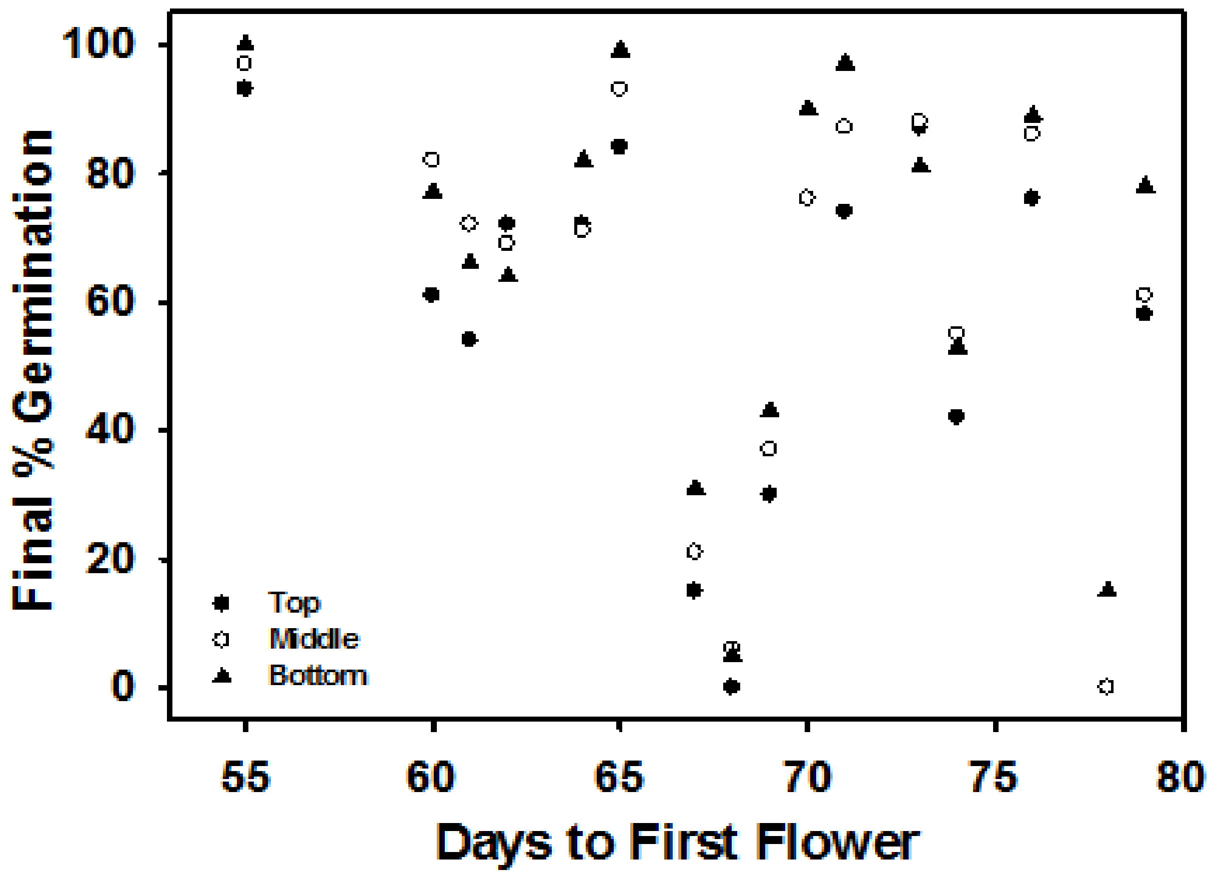

Figure 2.6 Final percent germination of kochia seed from the top, middle and bottom sections of plants corresponding to the days to first flower for the F2 generation in the greenhouse.

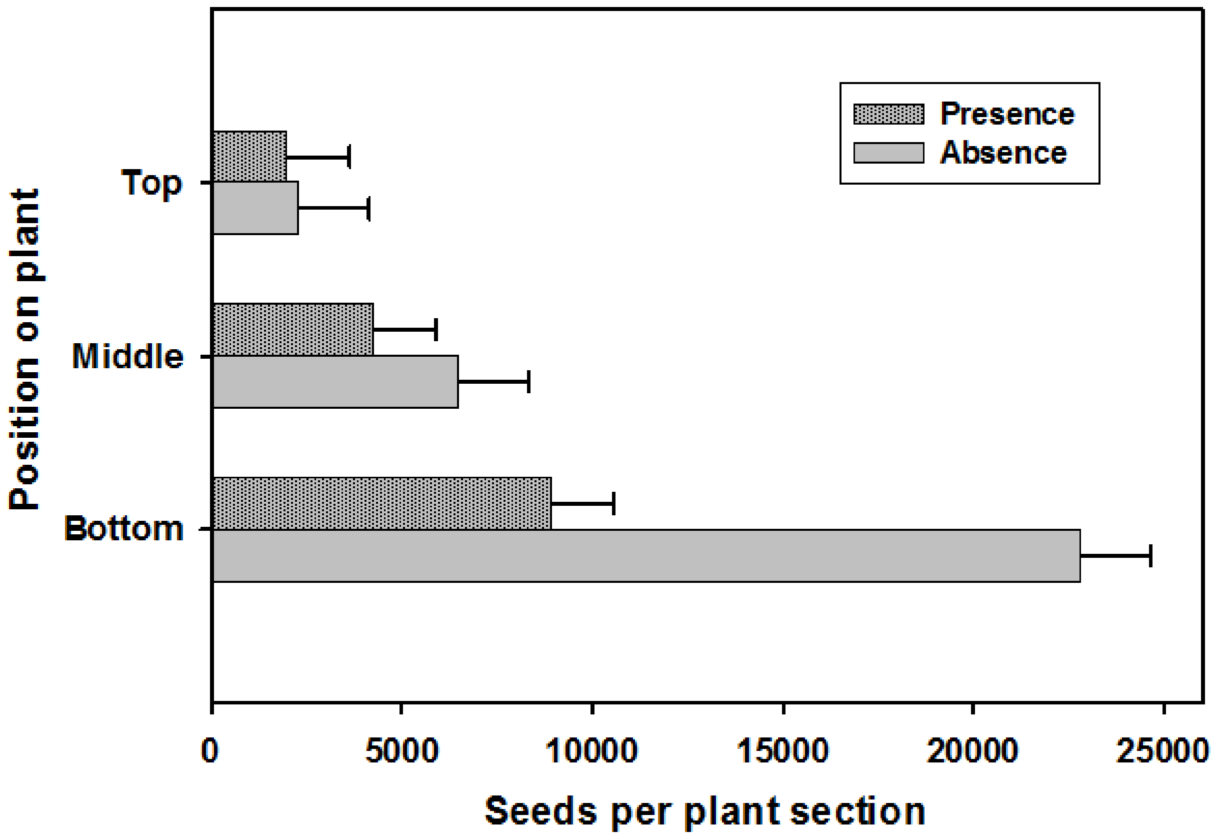

Figure 2.7 Seed production by position on plant from field-grown kochia in the presence and absence of a canopy at Hays, KS pooled across years.

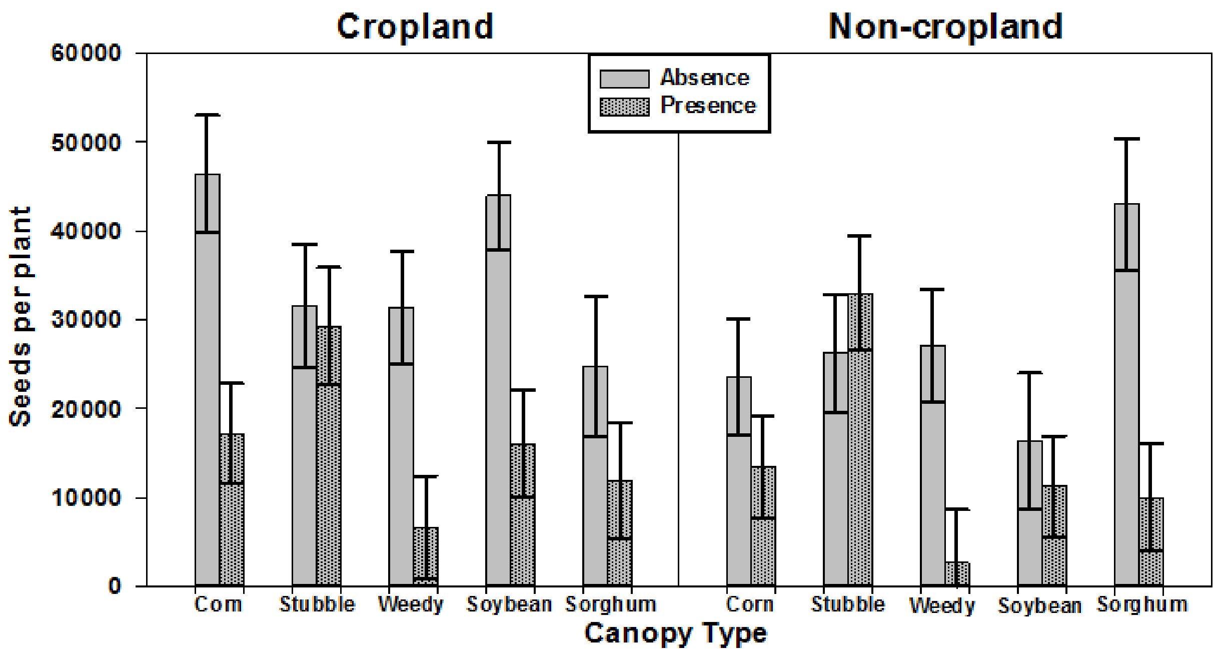

Figure 2.8 Seed production per plant in the presence and absence of a canopy for cropland and non-cropland biotypes at Hays, KS pooled across years.

Table 2.1 Final plant heights (SE) for the first cycle of self-pollination in the greenhouse with both biotypes and subsamples.

Biotype	Height (SE)
	cm
Cropland	134 (4.3)
Cropland subsample	236 (10.8)
Non-crop	107 (3.3)
Non-crop subsample	136 (12.5)
LSD (0.05)	31.2

Table 2.2 Final plant heights (SE) for the second cycle of self-pollinations in the greenhouse with cropland and non-cropland biotypes with non-cold treatment (NCT) and cold treatment (CT) parent seed.

	Height (SE)	
Biotype	NCT	CT
	-------------cm-------------	
Cropland	83 (3.4)	104 (3.2)
Non-cropland	92 (2.7)	92 (2.6)
LSD (0.05)	10.8	

Table 2.3 Final observed plant heights (SE) in the presence and absence of canopy and with both cropland and non-cropland biotypes at Hays, KS pooled across years.

	Height (SE)			
Canopy	Absence	Presence	Cropland	Non-cropland
	---------------------------------------cm---------------------------------------			
Corn	59 (3.3)	76 (3.2)	71 (3.2)	64 (3.3)
Stubble	45 (3.3)	57 (3.2)	53 (3.2)	49 (3.3)
Weedy	56 (3.4)	51 (3.3)	60 (3.2)	46 (3.4)
Soybean	53 (3.2)	58 (3.3)	56 (3.2)	55 (3.2)
Sorghum	57 (3.4)	75 (3.3)	63 (3.3)	69 (3.4)
LSD (0.05)	8.3		8.3	

Table 2.4 Kochia 1000-seed weights (SE) for cropland and non-cropland biotypes in the presence or absence of respective canopies at Hays, KS pooled across years. Means followed by the same letter were not different at LSD (0.05).

		1000-seed weight (SE)			
Canopy	Biotype	Absence		Presence	
		------------------------------g------------------------------			
Corn	Cropland	0.75 (0.10)	BCDEF	0.77 (0.10)	BCDE
	Non-crop	0.68 (0.10)	BCDEF	0.92 (0.10)	A
Stubble	Cropland	0.71 (0.10)	BCDEF	0.64 (0.10)	DEF
	Non-crop	0.80 (0.10)	ABCD	0.62 (0.10)	F
Weedy	Cropland	0.64 (0.10)	DEF	0.66 (0.10)	DEF
	Non-crop	0.72 (0.10)	BCDEF	0.64 (0.10)	DEF
Soybean	Cropland	0.63 (0.10)	EF	0.70 (0.10)	BCDEF
	Non-crop	0.64 (0.11)	CDEF	0.82 (0.10)	AB
Sorghum	Cropland	0.67 (0.11)	BCDEF	0.74 (0.10)	BCDEF
	Non-crop	0.76 (0.11)	ABCDEF	0.81 (0.10)	ABC
LSD (0.05)			0.16		

Table 2.5 Kochia 1000-seed weights (SE) by position on the plant from Hays, KS pooled across years.

Position	1000-seed weights (SE)
	g
Top	0.68 (0.10)
Middle	0.74 (0.10)
Bottom	0.73 (0.10)
LSD (0.05)	0.04

Table 2.6 Seed production per plant for cropland and non-cropland biotypes in the presence or absence of respective canopies at Hays, KS pooled across years. Means followed by the same letter were not different at LSD (0.05).

		Seeds per plant (SE)			
Canopy	Biotype	Absence		Presence	
Corn	Cropland	45,455 (6,625)	A	17,170 (5,635)	CDEF
	Non-crop	23,644 (6,486)	ABCDEF	13,454 (5,822)	DEF
Stubble	Cropland	31,577 (6,937)	ABCDE	29,240 (6,633)	ABCDE
	Non-crop	26,249 (6,623)	ABCDEF	33,000 (6,385)	ABCD
Weedy	Cropland	31,370 (6,355)	ABCD	6,629 (5,823)	EF
	Non-crop	27,162 (6,356)	ABCDE	2,663 (5,979)	F
Soybean	Cropland	43,971 (5,970)	AB	16,062 (5,973)	CDEF
	Non-crop	16,351 (7,638)	BCDEF	11,293 (5,635)	DEF
Sorghum	Cropland	24,796 (7,913)	ABCDEF	11,887 (6,494)	DEF
	Non-crop	43,023 (7,360)	ABC	10,036 (6,067)	DEF
LSD (0.05)			25,613		

Table 2.7 Percent germination and viability for NCT kochia seed in the presence and absence of a weedy canopy in 2012.

Canopy	Biotype	Germ % (SE)	Viability % (SE)
Absence	Non-crop	95 (7.1)	1 (4.7)
	Cropland	96 (5.2)	1 (3.6)
Presence	Non-crop	82 (4.5)	6 (3.2)
	Cropland	81 (4.1)	14 (2.9)
LSD (0.05)		8	5

Table 2.8 Percent germination of CT kochia seed in the presence and absence of weedy and grain sorghum canopies and percent viability in the presence and absence of a weedy canopy in 2012.

Canopy Type	Canopy	Biotype	Germ % (SE)	Viability % (SE)
Weedy	Absence	Non-crop	99 (5.6)	0 (4.0)
		Cropland	93 (3.9)	0 (2.9)
	Presence	Non-crop	82 (3.2)	5 (5.4)
		Cropland	77 (2.8)	12 (2.1)
Sorghum	Absence	Non-crop	87 (3.5)	
		Cropland	100 (4.0)	
	Presence	Non-crop	87 (3.5)	
		Cropland	91 (3.4)	
LSD (0.05)			7	3

Sources of Materials

[1] Metro-Mix 360 commercial potting mix, Sun-Gro Horticulture Canada Ltd., 770 Silver Street, Agawam, MA 01001.

[2] Miracle-Gro soluble fertilizer, Scotts Miracle-Gro Products Inc., 14111 Scottslawn Road, Marysville, OH 43041.

[3] South Dakota seedblower, Seedburo Equiptment Co., 2293 S. Mt. Prospect Road, Des Plaines, Il 60018.

[4] Monosem 6-row vacuum planter, Monosem Inc., 1001 Blake Street, Edwardsville, KS 66111.

[5] Laponite RD® Hydrous sodium lithium magnesium silicate, Unit 2 Ashville Way Off Watlington Road, Cowley, Oxford OX4 6TU.

[6] SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513-2414.

[7] SigmaPlot Systat Software Inc., 1735 Technology Drive, Suite 430, San Jose, CA 95110.

Chapter 3 - Response of Kochia Biotypes to Glyphosate, Atrazine, and Chlorsulfuron

ABSTRACT

Kochia in Kansas has a history of herbicide-resistance to multiple modes of action. Therefore, greenhouse experiments were conducted to determine the response of kochia biotypes used in greenhouse and field experiments to glyphosate, atrazine, and chlorsulfuron. Kochia seeds were collected in 2011 from cropland and non-cropland locations in Hays, KS and from Stockton, KS. Those seeds were used in 2012 and 2013 in field experiments in Hays, KS. The cropland and non-cropland biotypes were also used in a greenhouse experiment which included two cycles of self-pollination (F1 and F2 generations). Two treatments were imposed on seed from the F2 generation, which were non-cold treatment (NCT) and cold treatment (CT). The herbicide screen was conducted once with four treatments: glyphosate, atrazine, chlorsulfuron, and a no herbicide control in a randomized complete block design with four to five replications. Field rates used were 840 g ae/ha for glyphosate, 2.24 kg ai/ha for atrazine, and 18g ai/ha for chlorsulfuron and were applied when plants were 4 to 22 cm in height. A separate growth analysis was conducted on five plants from each biotype on the same day and 21 days after treatment (DAT). The only significant difference in the growth analysis was at 21 DAT where the weedy biotype from Stockton had less leaf area and leaf weight compared to the cropland biotype from the first year of the field experiment. Cropland and non-cropland biotypes from both years of the field experiment had 0 to 40% injury with glyphosate. Atrazine caused 100% injury to F2 plants from the NCT for both biotypes. Injury ratings to chlorsulfuron varied between biotypes with the cropland biotypes having 0 to 20% injury for all plants while non-cropland biotypes had 70 to 100% injury for all biotypes. This experiment is an indication of the variability in kochia and shows the differences among and within biotypes for susceptibility to field rates of glyphosate, atrazine, and chlorsulfuron.

INTRODUCTION

Kochia (*Kochia scoparia* (L.) Schrad) is an annual broadleaf weed found throughout crop and ruderal areas of the Canadian Prairies and the Great Plains of the United States (Wiese 1970; Nussbaum et al. 1985; Leeson et al. 2005; Friesen et al. 2009). It is a member of the *Chenopodiaceae* or goosefoot family, native to Eurasia and was introduced to the Americas in the mid to late 1800's as an ornamental that subsequently escaped cultivation and formed naturalized populations (Georgia 1914; Scoggan 1957). Kochia is a C_4 summer annual herbaceous dicot plant that thrives in hot and dry environments due to its ability to germinate at low soil temperatures, grow rapidly (Evetts and Burnside 1972; Schwinghamer and Van Acker 2008), and its tolerance to heat, drought (Pafford and Wiese 1964; Coxworth et al. 1969), and saline soils (Weatherspoon and Schwiezer 1970).

The ability of kochia to develop herbicide resistance so quickly and easily when compared to other weeds is possibly a function of several factors. Kochia has difficult-to-wet leaf characteristics that may decrease herbicide efficacy (Friesen et al. 2009). Kochia leaves are generally pubescent with crystalline epicuticular wax, which may result in suspended herbicide spray droplets above the cuticle and, consequently reduced absorption (Harbour et al. 2003). Growth stage and herbicide dose markedly affect herbicide efficacy with a decline in control as kochia size increases (Friesen et al. 2009). Kochia has high genetic diversity among and within populations which is a contributing factor for selection of herbicide-resistant individuals (Mengistu and Messersmith 2002), as well as wind-mediated pollen movement and protogynous flowering which results in a high degree of outcrossing (Guttieri and Eberlein 1998), and leads to increasingly more herbicide-resistant populations of this species (Thompson et al. 1994).

Kochia has developed resistance to herbicides with different modes of action, including acetolactate synthase (ALS) inhibitors, EPSPS (5-enolpyruvyl-shikimate-3-phosphate synthase) inhibitors, synthetic auxins, and photosystem II inhibitors (Peterson 1999; Thompson et al. 1994; Waite et al. 2013; Heap 2014). Herbicide-resistant kochia, specifically to triazines in cultivated fields, first appeared in Kansas in 1976 (Peterson 1999). Triazine-resistance in kochia is usually is endowed by a point mutation in the chloroplast *psb*A gene, resulting in a substitution of glycine for serine at residue 264 (Mengistu et al. 2005). Similarly, ALS-inhibitor resistance in kochia usually is endowed by a single point mutation in the ALS nuclear gene and is inherited as a dominant or semidominant trait.and is controlled by a single nuclear gene (Primiani et al. 1990). Chlorsulfuron resistant kochia was confirmed in Kansas wheat fields in 1987 (Thompson et al. 1994). More recently, in 2007, glyphosate resistant kochia was confirmed in Kansas in multiple cropping systems such as corn, cotton and soybeans (Waite et al. 2013). Resistance to glyphosate is kochia is from gene amplification of EPSPS (Wiersma 2012).

Knowing that kochia in Kansas has a history of herbicide-resistance to multiple modes of action the objective for this study was to screen multiple kochia biotypes for response to field use rates of glyphosate, atrazine, and chlorsulfuron.

MATERIALS AND METHODS

Kochia seed were collected in 2011 from a cropland and non-cropland source originating from Hays, KS and were used in both greenhouse and field experiments (from Chapter 2). Cropland and non-cropland biotypes were used in the field experiment in 2012 (Y1). From the field experiment in 2012 one plant from each of the cropland and non-cropland biotypes was chosen for the field experiment in 2013 (Y2). Kochia seeds were collected in 2011 from Stockton, KS and were used as the weedy biotype in both Y1 and Y2 of the field experiment for a total of five biotypes to screen from the field experiment.

In the greenhouse experiment cropland and non-cropland kochia biotypes underwent two cycles of self-pollination to generate F1 and F2 seed generations. From the F1 seed generation two kochia plants from each biotype, with varying days to first flower, were chosen for a total of four biotypes. Plants producing seed for the F2 generation in the greenhouse were from both biotypes and from two seed treatments non-cold treatment (NCT) and cold treatment (CT). NCT seeds were taken directly off the plant and the CT seeds were placed in a freezer set at -4 C for six weeks to simulate an overwintering period. Seed from one plant was chosen from each biotype with each seed treatment for a total of eight biotypes from the greenhouse experiment to screen.

All kochia plants were grown in a greenhouse with a 12:12 day/night cycle at 27:22 ±2 C, respectively and watered as needed. All 13 seed biotypes were placed on the soil surface in separate 28 by 19 by 6 cm flats filled with 480g of a commercial growing medium for germination on January 13, 2014. Pots measuring 8.5 by 8.5 by 7 cm were filled with 85 g of the same growing medium and single kochia seedlings were transplanted from the flats when plants were 3 to 5 cm tall. Cropland and non-cropland biotypes from Y1 and Y2 of the field experiment and the F2 non-cropland biotype with NCT were transplanted on January 31, 2014. The weedy biotype from the field experiment and the F2 cropland biotype with NCT from the greenhouse were transplanted on February 10, 2014. (Insufficient seedlings were recruited from the other biotypes to conduct the study.)

This experiment was conducted once with four treatments: glyphosate, atrazine, chlorsulfuron, and a no herbicide control with four to five replications. The herbicide sources for glyphosate, atrazine, and chlorsulfuron were Roundup WeatherMax, Aatrex 4L, and Glean, respectively. All glyphosate applications included 10.2 g/L AMS, while all atrazine applications included 1% (v/v) crop oil concentrate and all chlorsulfuron applications included 0.25% non-ionic surfactant. All herbicides were applied at a typical field use rate: 840 g ae/ha for glyphosate, 2.24 kg ai/ha for atrazine, and 18g ai/ha for chlorsulfuron. Treatments were applied with a bench-type sprayer using a Teejet 8002E flat fan nozzle calibrated to deliver 168 L/ha at 222 kPa with a speed of 3.22 km/h when plants were 4 to 22 cm in height. Visible control symptoms and mortality rating were determined at 7, 14, and 21 days after treatment (DAT) on a scale of 0 to 100%, where 0 equals no control and 100 equals complete injury or mortality compared to the untreated control. Plants that had injury ratings of greater than 80% were classified as susceptible to the herbicide treatment and all other remaining plants were considered survivors. Survivors at 21 DAT had their injury ratings averaged within their respective kochia biotype. Final fresh weights for all treated plants were recorded and plants were placed in paper bags in an oven at 60 C for 7 days to dry and dry weights were recorded. A growth analysis was conducted on three to five plants per biotype, at time of treatment (TOT) and at 21 DAT. Data collected included, plant height (cm), leaf area (cm^2), fresh leaf weight (g), fresh stem weight (g), and total leaf and stem dry weight (g). Growth analysis data at TOT and 21 DAT were analyzed using PROC MIXED across biotypes. Graphs were generated using SigmaPlot v. 12.

RESULTS AND DISCUSSION

Most biotypes produced enough kochia seedlings to conduct both the screen and growth analysis studies. Insufficient seedlings were generated by any of the four seed sources from the F1 generation or from the two seed sources from the cropland and non-cropland CT seeds of the

F2 generation to conduct the herbicide screen and growth analysis. Also, only 21 plants were available from the NCT cropland biotype from the F2 generation in the greenhouse with only three plants being used for the control and both TOT and 21 DAT of the growth analysis and four plants for each herbicide treatment.

Plants from the F2 generation of the non-cropland biotype with the NCT had the quickest rate of growth and were treated at 36 days after planting (DAP). All cropland and non-cropland biotypes used in the field experiment were treated at 43 DAP, followed by the weedy biotype from Stockton and the cropland biotype from the F2 generation NCT being treated at 46 DAP.

Although results varied for the growth analysis at time of treatment there were no significant differences for plant height, leaf area, fresh leaf weight, fresh stem weight, and dry leaf and stem weight across all biotypes (Table B.1). Similar results were seen for the growth analysis at 21 DAT with no significant differences seen for plant height, stem weight and dry biomass across biotypes (Table B.2). There was a difference at 21 DAT for plants from the weedy biotype from Stockton, KS which produced plants with less total leaf area and fresh leaf weight compared to the Y1 cropland biotype (Table 3.1).

There was variability with individual plant response to field rates of glyphosate, atrazine and chlorsulfuron among and within biotypes. The Y1 cropland and non-cropland biotypes showed similarities in percent injury to glyphosate and atrazine. Glyphosate injury ranged between 0 and 40% for all plants, while atrazine injury ranged between 15 and 50% for 80% of plants for both biotypes (Figure 3.1). The other 20% of plants from both biotypes showed between 90% and 100% injury to atrazine (Figure 3.1). The injury response to chlorsulfuron varied between biotypes with the cropland biotype only exhibiting 0 to 20% injury across all plants while all non-cropland plants were susceptible and had between 90 and 100% injury (Figure 3.1).

Results from the Y2 cropland and non-cropland seeds at 21 DAT showed similar results to those from the Y1 biotypes at 21 DAT. The injury response of both Y2 cropland and non-cropland biotypes to glyphosate ranged from 0 to 25% for all plants (Figure 3.2). Injury responses to atrazine were mixed within biotypes and ranged from 30 to 75% in the cropland biotype and 20 to 50% in the non-cropland biotype (Figure 3.2). The second year of seed from field-grown kochia showed similar injury ratings to chlorsulfuron with the cropland biotype only exhibiting between 10 to 15% across all plants while in the non-cropland biotype all plants had between 75 and 100% injury with 60% of plants showing a rating of 100% (Figure 3.2). The weedy biotype from Stockton had mixed injury to glyphosate with 40% of plants having 40% injury, 40% of plants having 75 and 80% injury, and 20% having 100% injury (Figure 3.3). All plants in the weedy biotype were susceptible to atrazine and exhibited injury between 90 and 100% (Figure 3.3). Chlorsulfuron injury to the weedy biotype was minimal with 60% of plants having between 0 and 5% injury and the other 40% having 25% injury (Figure 3.3).

Results from the F2 generation cropland and non-cropland biotypes with the NCT seed treatment exhibited varied injury at 21 DAT within biotypes to glyphosate. The cropland biotype had 75% of plants showing between 20 and 30% injury and 25% of plants had 65% injury, while the non-cropland biotype had 60% of plants exhibiting between 10 and 25% injury and the other 40% were susceptible showing 100% injury (Figure 3.4). All plants from both biotypes had 100% injury from a field rate of atrazine (Figure 3.4). Injury from chlorsulfuron varied between biotypes with there being 0% injury for all plants from the cropland biotype and 70 to 100% injury for all plants in the non-cropland biotype (Figure 3.4).

The results from the growth analysis showed biotype differences with Y1 cropland seed from Hays having greater leaf area and leaf weight compared to the weedy biotype from Stockton. This is due to those biotypes having different genetic backgrounds as a result of being collected from different geographic locations. The plant injury ratings show that in general, both cropland and non-cropland biotypes from the field experiment had 100% survival to a field rate of glyphosate. In Y1 both cropland and non-cropland biotypes had 80% survival to a field rate of atrazine with plants that survived having average injury ratings at 36 (7.2) and 39% (4.3), respectively (Table 3.2). In Y2 both cropland and non-cropland biotypes had 100% survival to a field rate of atrazine with plants that survived having average injury ratings at 51 (7.3) and 29% (5.6), respectively (Table 3.2). Differences were seen between field biotypes to a field rate of chlorsulfuron, with the cropland biotype having 100% survival both years and the non-cropland biotype having 0% survival in Y1 and 20% survival in Y2. The weedy biotype had 60% survival to glyphosate, 0% survival to atrazine and 100% survival to chlorsulfuron. The F2 generation with the NCT seed showed varied injury ratings for both biotypes to a field rate of glyphosate. All plants in the F2 generation with NCT seed had 100% injury to atrazine. Atrazine resistant kochia has a fitness penalty compared to atrazine susceptible kochia so results from the F2 generation can be attributed to those biotypes being the most fit plants from each biotype meaning plants that would have been less susceptible to atrazine wouldn't have been chosen. In the F2 generation the cropland biotype had 100% survival to a field rate of chlorsulfuron, while the non-cropland biotype only had 20% survival. This experiment is an indication of the variability of kochia susceptibility to field rates of glyphosate, atrazine, and chlorsulfuron across and within biotypes.

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Figures and Tables

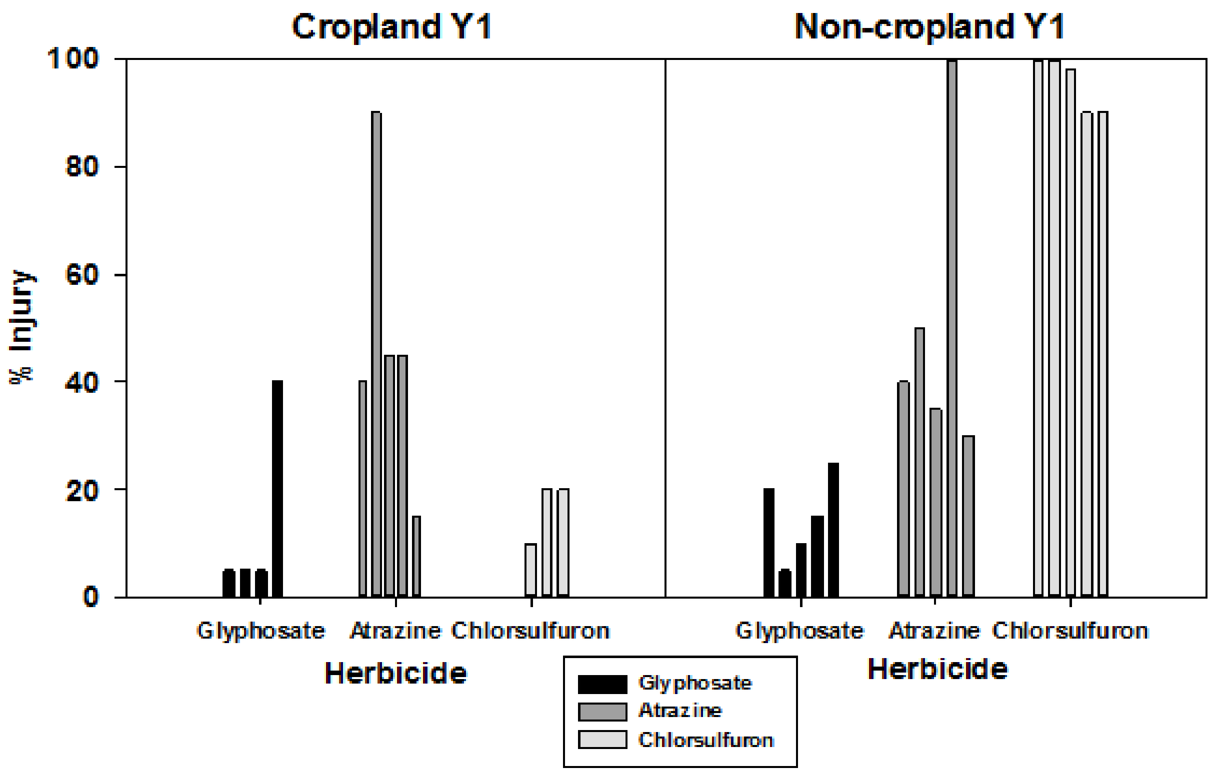

Figure 3.1 Individual plant response (% injury relative to untreated control) at 21 DAT from field rate application of glyphosate, atrazine, and chlorsulfuron to Y1 cropland and non-cropland biotypes from Hays, KS.

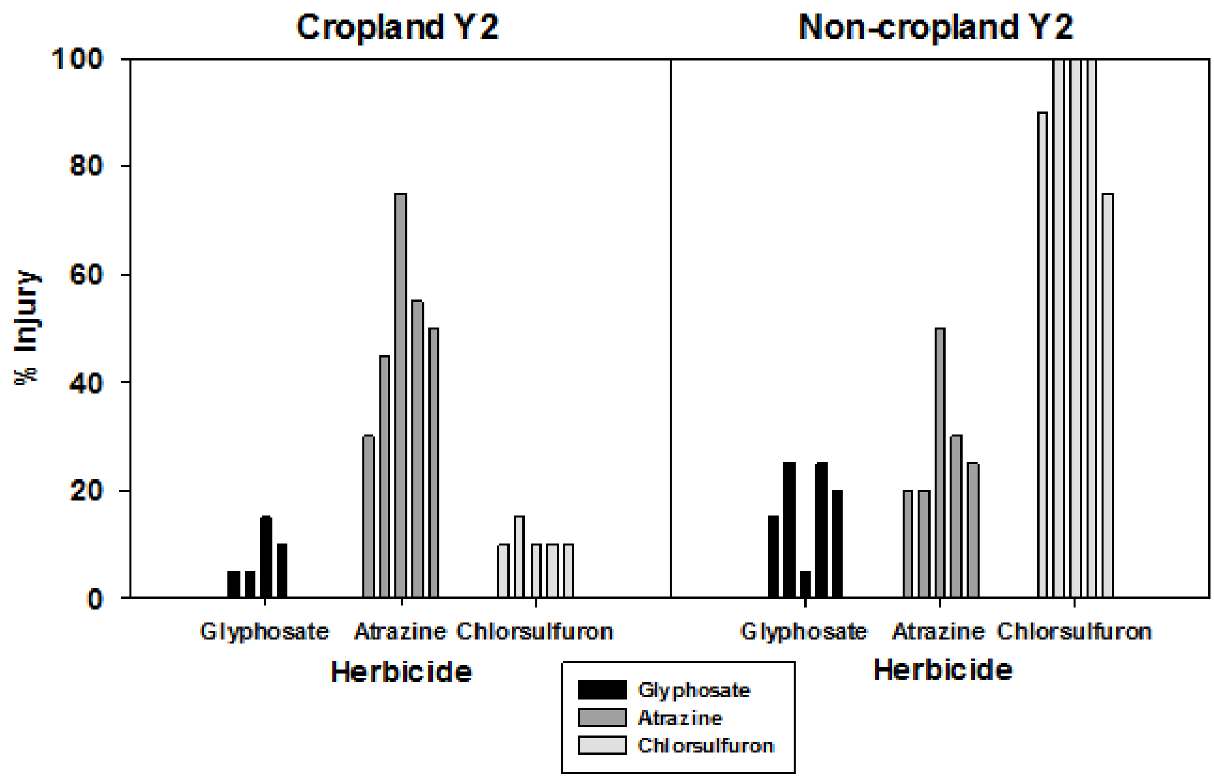

Figure 3.2 Individual plant response (% injury relative to untreated control) at 21 DAT from field rate application of glyphosate, atrazine, and chlorsulfuron to Y2 cropland and non-cropland biotypes from Hays, KS.

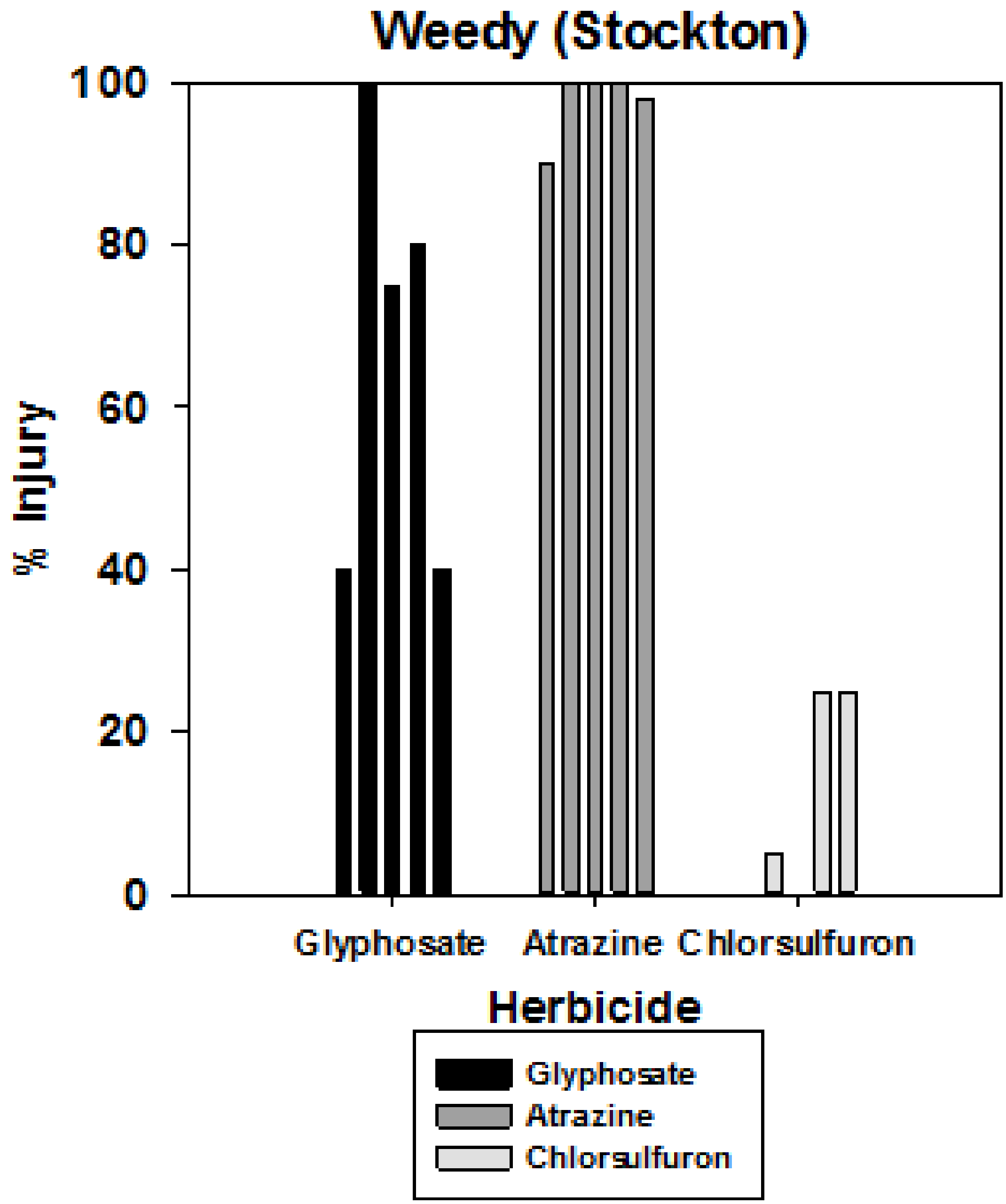

Figure 3.3 Individual plant response (% injury relative to untreated control) at 21 DAT from field rate application of glyphosate, atrazine, and chlorsulfuron to weedy biotype from Stockton, KS.

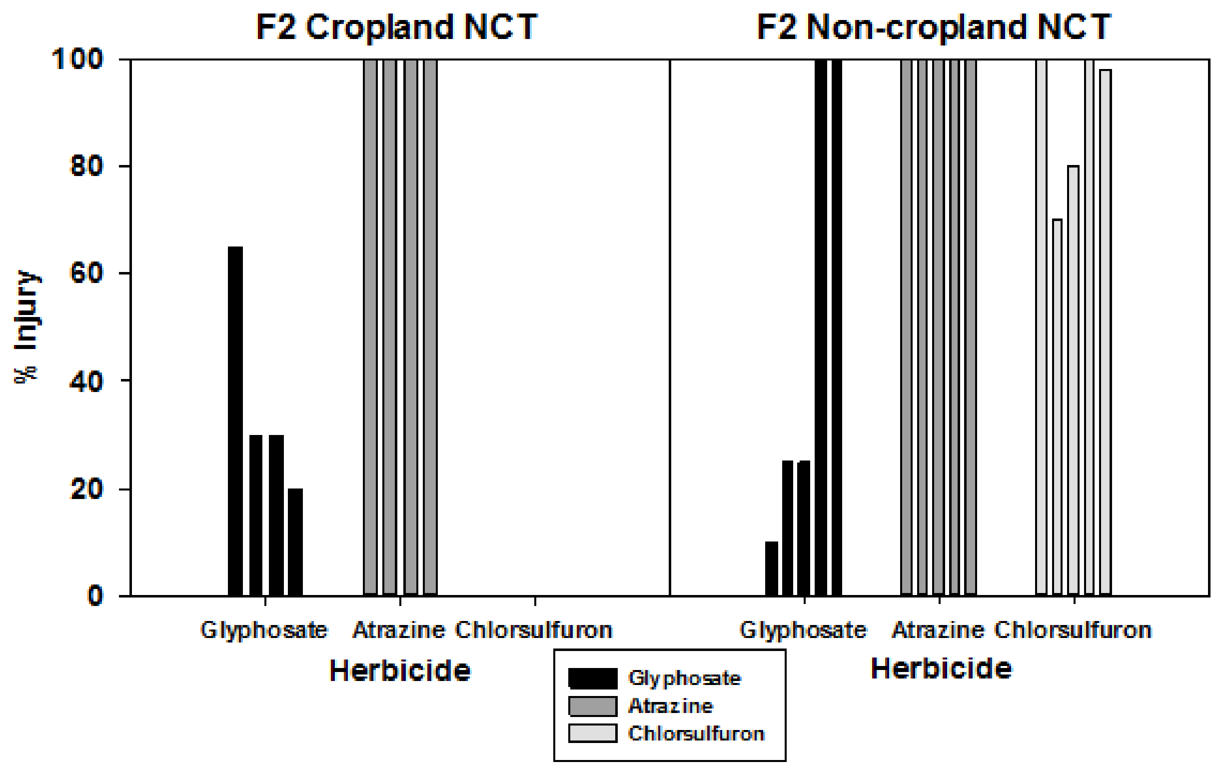

Figure 3.4 Individual plant response (% injury relative to untreated control) at 21 DAT from field rate application of glyphosate, atrazine, and chlorsulfuron to F2 generation of cropland and non-cropland biotypes with the non-cold treatment (NCT) parent seed.

Table 3.1 Growth analysis (SE) results for leaf area (cm^2) and fresh leaf weight (g per plant) for all biotypes at time of treatment (TOT) and at 21 days after treatment (DAT). Means followed by the same letter within a column were not different at LSD (0.05).

	Leaf Area				Leaf Weight			
Kochia Biotype	TOT		21 DAT		TOT		21 DAT	
	---cm^2---				---g---			
Cropland	107.25 (19.94)	A	245.69 (25.35)	A	3.08 (0.54)	A	6.50 (0.71)	A
Non-crop	82.62 (19.94)	A	200.60 (25.35)	AB	2.25 (0.54)	A	5.11 (0.71)	AB
Cropland Y2	132.71 (19.94)	A	226.50 (25.35)	AB	3.72 (0.54)	A	6.78 (0.71)	AB
Non-crop Y2	81.54 (19.94)	A	143.12 (25.35)	AB	2.19 (0.54)	A	3.94 (0.71)	AB
Weedy	71.86 (19.94)	A	124.98 (25.35)	B	1.96 (0.54)	A	3.43 (0.71)	B
F2 Cropland NCT	114.08 (25.74)	A	117.66 (32.73)	AB	3.15 (0.69)	A	3.35 (0.92)	AB
F2 Non-crop NCT	86.33 (19.94)	A	183.89 (25.35)	AB	2.44 (0.54)	A	4.82 (0.71)	AB
LSD (0.05)	94.24		119.80		2.54		3.37	

Table 3.2 Percent survival and average % injury (SE) for plants that survived a field rate application of glyphosate, atrazine, and chlorsulfuron compared to untreated control for all biotypes at 21 DAT.

	Visual response					
	Glyphosate		Atrazine		Chlorsulfuron	
Kochia Biotype	Survival	Injury	Survival	Injury	Survival	Injury
	----------%					-----------
Cropland Y1	100	11 (7.3)	80	36 (7.2)	100	10 (4.8)
Non-crop Y1	100	15 (3.5)	80	39 (4.3)	0	
Cropland Y2	100	7 (2.5)	100	51 (7.3)	100	11 (1.0)
Non-crop Y2	100	18 (3.7)	100	29 (5.6)	20	75 (0.0)
Weedy	60	52 (11.7)	0		100	11 (5.8)
F2 Cropland NCT	100	36 (9.9)	0		100	0 (0)
F2 Non-crop NCT	60	20 (5.0)	0		20	70 (0)

Appendix A - Weather Data

Table A.1 Mean temperatures and precipitation for both years of the field experiment at Hays, KS.

	2012		2013		30-year average
Month	Precipitation	Mean Temperature	Precipitation	Mean Temperature	Precipitation
	mm	C	mm	C	mm
Janurary	0.8	0.9	19.3	-0.9	12.7
February	32.3	1.6	30.2	0.2	17.8
March	35.6	12.3	19.8	4.6	46.0
April	72.9	14.7	26.9	9.3	54.1
May	40.4	20.8	54.9	18.2	82.8
June	21.6	26.4	69.3	24.7	72.1
July	5.6	29.8	179.8	25.6	98.0
August	85.6	24.8	15.0	25.0	77.2
September	27.2	20.1	75.7	22.3	52.1
October	23.9	11.2	25.1	12.2	40.6
November	0.0	6.6	29.5	4.4	23.6
December	19.8	0.1	1.3	-2.0	18.3
Total	365.7		546.8		595.3

Appendix B - Kochia Plant Heights from Hays, KS

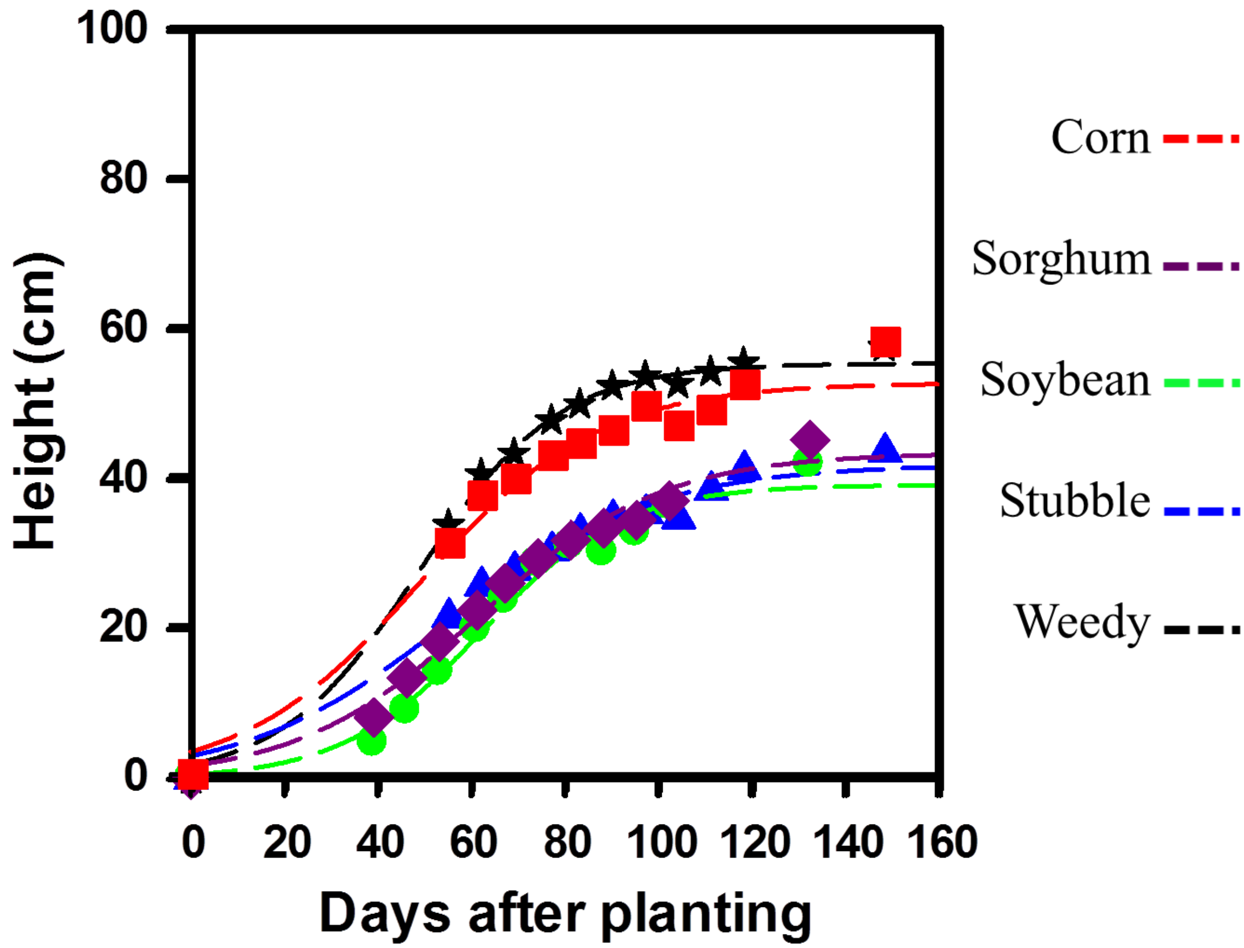

Figure B.1 Observed kochia heights (points) and regression curves (lines) in the absence of canopy types in 2012 at Hays, KS (parameter estimates Table B.1)

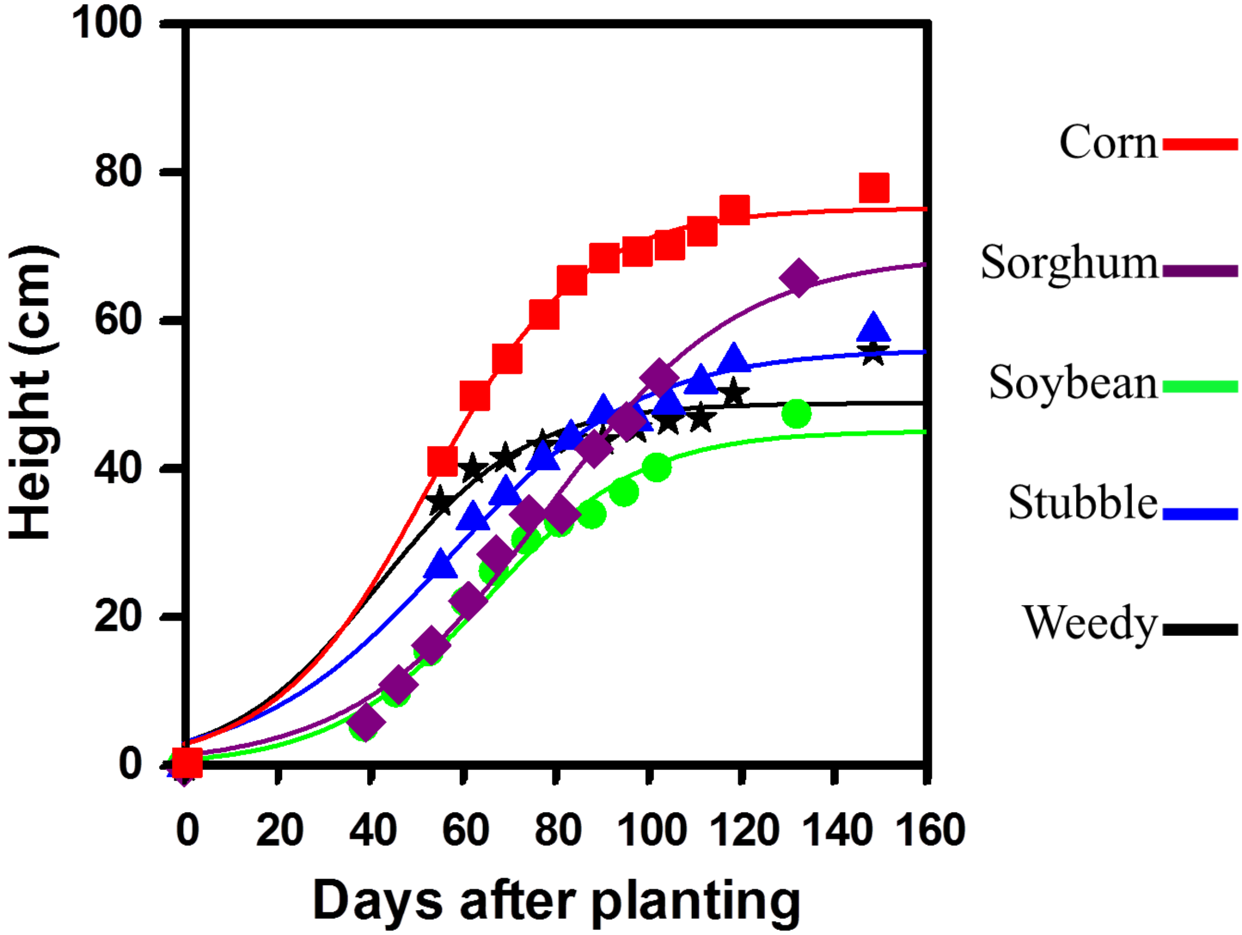

Figure B.2 Observed kochia heights (points) and regression curves (lines) in the presence of canopy types in 2012 at Hays, KS (parameter estimates Table B.1)

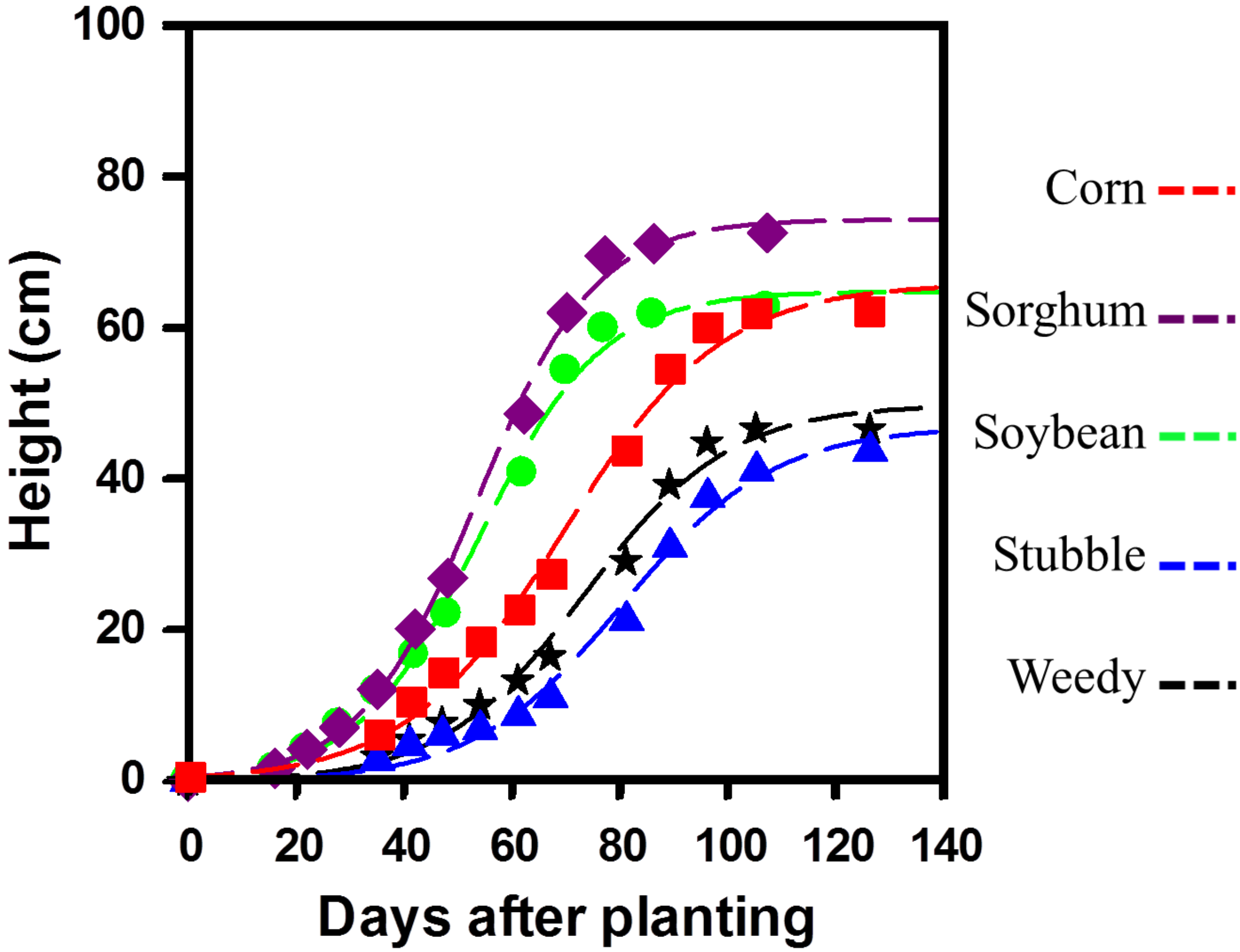

Figure B.3 Observed kochia heights (points) and regression curves (lines) in the absence of canopy types in 2013 at Hays, KS (parameter estimates Table B.2)

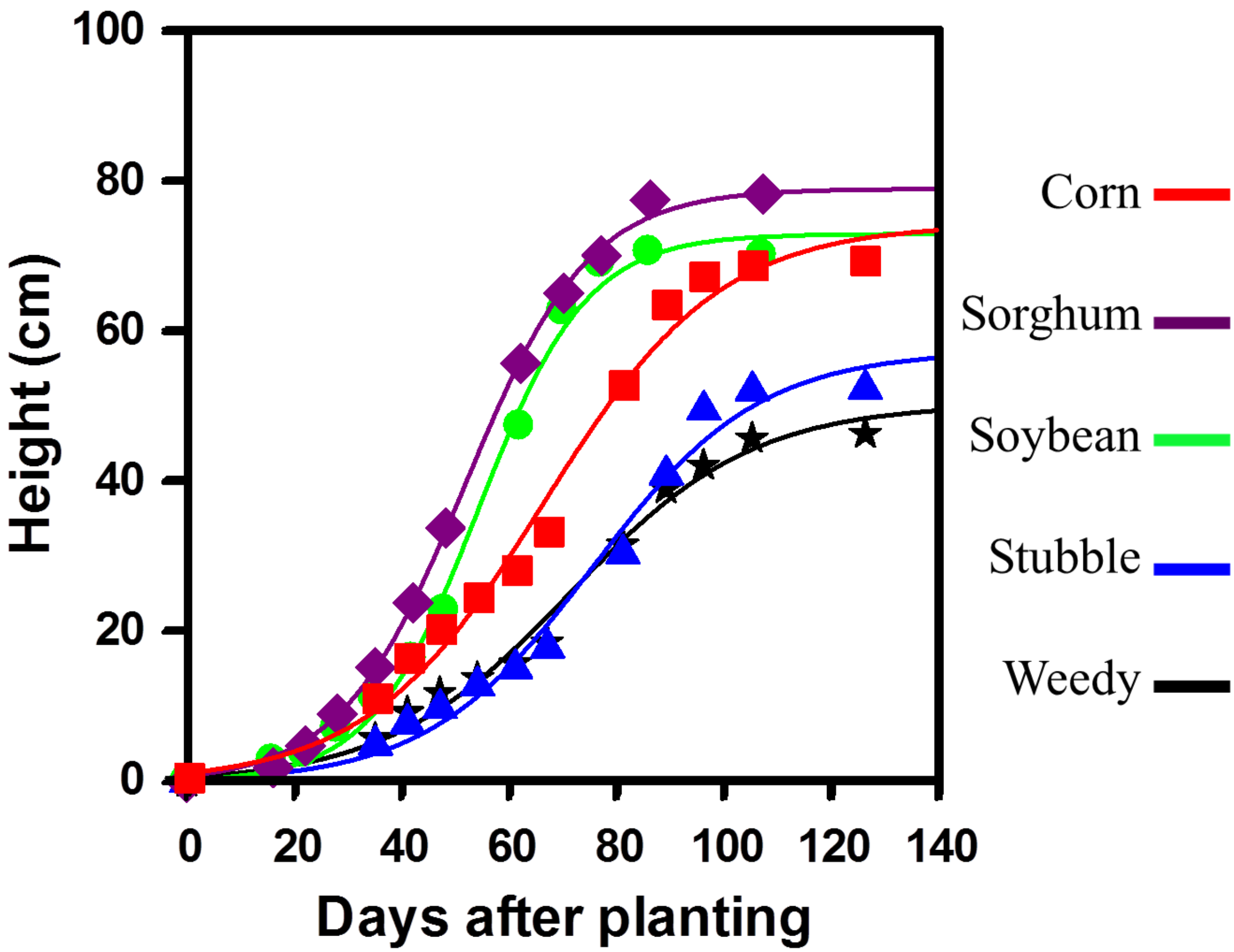

Figure B.4 Observed kochia heights (points) and regression curves (lines) in the presence of canopy types in 2013 at Hays, KS (parameter estimates Table B.2)

Table B.1 Parameter estimates (SE) for kochia height in the absence and presence of canopy types in 2012 at Hays, KS as seen in Figure B.1 and B.2. Equation: Ht = [a/ 1 + $(x/x_o)^b$] where Ht = kochia height (cm), x = days after planting canopy type, a = maximum height, b = slope of curve at inflection point and x_o = days after planting at inflection point.

		Parameter estimates			
Canopy	P/A	a	b	X_o	r^2
Corn	Presence	75 (2.48)	16.5 (3.26)	52 (2.66)	0.80
	Absence	53 (2.66)	19.1 (5.01)	49 (4.49)	0.64
Stubble	Presence	56 (3.93)	20.8 (5.87)	57 (4.61)	0.59
	Absence	42 (2.60)	21.9 (5.38)	55 (4.36)	0.64
Weedy	Presence	49 (1.99)	15.9 (4.76)	42 (5.97)	0.60
	Absence	56 (2.49)	15.0 (5.05)	49 (4.76)	0.61
Soybean	Presence	45 (3.31)	16.7 (3.07)	65 (3.68)	0.70
	Absence	39 (2.54)	15.1 (2.76)	62 (3.16)	0.72
Sorghum	Presence	69 (7.83)	20.5 (5.87)	78 (5.97)	0.71
	Absence	44 (4.72)	19.6 (5.20)	62 (3.16)	0.62

Table B.2 Parameter estimates (SE) for kochia height in the absence and presence of canopy types in 2013 at Hays, KS as seen in Figure B.3 and B.4. Equation: Ht = [a/ 1 + $(x/x_o)^b$] where Ht = kochia height (cm), x = days after planting canopy type, a = maximum height, b = slope of curve at inflection point and x_o = days after planting at inflection point.

		Parameter estimates			
Canopy	P/A	a	b	X_o	r^2
Corn	Presence	74 (7.12)	16.4 (3.76)	67 (5.11)	0.62
	Absence	66 (5.87)	14.7 (3.12)	70 (4.48)	0.66
Stubble	Presence	58 (3.59)	15.5 (1.94)	76 (3.07)	0.84
	Absence	47 (2.40)	14.0 (1.35)	81 (2.21)	0.90
Weedy	Presence	51 (2.41)	17.1 (1.68)	72 (2.53)	0.90
	Absence	50 (4.54)	13.4 (2.82)	74 (4.27)	0.70
Soybean	Presence	73 (1.80)	10.1 (0.72)	55 (1.07)	0.95
	Absence	65 (3.53)	11.3 (1.63)	54 (2.44)	0.85
Sorghum	Presence	79 (4.14)	11.5 (1.69)	52 (2.43)	0.83
	Absence	75 (4.09)	10.9 (1.67)	54 (2.46)	0.84

Appendix C - Growth Analysis

Table C.1 Growth analysis (SE) at time of treatment for all biotypes.

Kochia Biotype	Height	Leaf Area	Leaf Weight	Stem Weight	Dry Biomass
	cm	cm^2	-----------------------------	g	-----------------------------
Cropland Y1	16 (1.9)	107.25 (19.94)	3.08 (0.54)	1.07 (0.21)	0.50 (0.10)
Non-crop Y1	13 (1.9)	82.62 (19.94)	2.25 (0.54)	0.72 (0.21)	0.37 (0.10)
Cropland Y2	18 (1.9)	132.71 (19.94)	3.72 (0.54)	1.24 (0.21)	0.59 (0.10)
Non-crop Y2	14 (1.9)	81.54 (19.94)	2.19 (0.54)	0.83 (0.21)	0.48 (0.10)
Weedy	13 (1.9)	71.86 (19.94)	1.96 (0.54)	0.60 (0.21)	0.26 (0.10)
F2 Cropland NCT	15 (2.4)	114.08 (25.74)	3.15 (0.69)	1.09 (0.27)	0.50 (0.13)
F2 Non-crop NCT	13 (1.9)	86.33 (19.94)	2.44 (0.54)	0.69 (0.21)	0.30 (0.10)

Table C.2 Growth analysis (SE) at 21 days after treatment for all biotypes.

Kochia Biotype	Height	Leaf Area	Leaf Weight	Stem Weight	Dry Biomass
	cm	cm^2	-----------------------------	g	-----------------------------
Cropland Y1	48 (6.3)	245.69 (25.35)	6.50 (0.71)	4.25 (0.61)	2.58 (0.36)
Non-crop Y1	41 (6.3)	200.60 (25.35)	5.11 (0.71)	3.13 (0.61)	1.91 (0.36)
Cropland Y2	52 (6.3)	226.50 (25.35)	6.78 (0.71)	4.56 (0.61)	2.91 (0.36)
Non-crop Y2	42 (6.3)	143.12 (25.35)	3.94 (0.71)	3.01 (0.61)	1.73 (0.36)
Weedy	36 (6.3)	124.98 (25.35)	3.43 (0.71)	2.55 (0.61)	1.44 (0.36)
F2 Cropland NCT	48 (8.1)	117.66 (32.73)	3.35 (0.92)	2.69 (0.79)	1.64 (0.46)
F2 Non-crop NCT	50 (6.3)	183.89 (25.35)	4.82 (0.71)	3.27 (0.61)	1.97 (0.36)